THE APPLICATION OF THE SHPOL'SKII EFFECT IN LOW TEMPERATURE LUMINESCENCE SPECTROMETRY

by

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Abstract

The application in Analytical Chemistry of the luminescence at low temperature in frozen solutions of organic compounds, in particular polycyclic aromatic hydrocarbons, in matrices that produce the Shpol'skii effect has been examined. A search of other possible matrices has been undertaken and a new solvent matrix, tetrahydrofuran (THF) has been shown to be suitable for production of quasi-linear emission spectra for a number of molecules. Some parameters affecting the quasi-linear luminescence signal, e.g.: presence of impurity, source of excitation used, have been examined. Improvements were made to the sample system used and a new sample cell which provides illumination and viewing conditions favourable for the study and analytical application of the Shpol'skii effect has been constructed and evaluated.

A wide range of compounds have been studied in THF ranging from several polycyclic aromatic hydrocarbons to aflatoxins, vitamins and alkaloids.

Several methods of signal registration have been applied to the quasi-linear luminescence method of analysis; systems using constant time, constant charge, photon counting and signal averaging techniques have been employed in these studies.

Dedication

I dedicate this thesis to my parents

Acknowledgements

The research described in this thesis is entirely original except where due reference is made and no part has previously been submitted for any other degree. The work was carried out in the Department of Chemistry of the Imperial College of Science and Technology between October 1972 and August 1975.

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Chapter I

Introduction

A major distinction between the technique of atomic absorption or emission spectrometry, and molecular absorption or emission spectrometry in condensed phases, using the electronic spectra in the visible or UV region is based on the different type of spectra observed in each. In the first case sharp atomic line spectra are observed and confer high specificity on the techniques; in molecular spectroscopy broad band spectra are observed which do not permit use of diffuseness which prevents the determination of peak wavelength for specific qualitative identification of absorbing or emitting species and give rise to overlap between spectra even in simple mixtures of compounds.

The broadness in electronic spectra is related to the simultaneous vibrational and rotational excitation which occurs on absorption of radiation.

Much of the present understanding of the structure of matter and the interaction of electro-magnetic energy with matter comes from spectroscopy, i.e. the study of the characteristic frequencies at which atoms and molecules absorb or emit radiation. However for complex species and in the UV and visible region of the electro-magnetic spectrum, very narrow resonance lines are difficult to obtain. Narrow line spectra are obtained for gases at low pressure where interparticle collisions, which broaden the spectral line width, are governed by the pressure and random motion of the atoms or molecules of gas.

In the case of a crystal lattice of a pure solid compound or of a lattice containing an impurity the energy levels are strongly influenced by the vibrations of the crystal lattice (phonons). A possible way to reduce this vibration is the reduction of temperature of the system being studied.

Historical Observations

Until 1900 the history of luminescence observations has been well described by E.N. Harvey.[1] Modern scientific studies started in 1888 when Eilhardt Wiedemann a German physicist, reported in a detailed paper[2] that quinine, esculin and many dye stuffs, absorbed in gelatin, phosphoresce more strongly at -80°C than at room temperature. At the same time the term "luminescenz" was born. In 1894, J. Dewar[3] made an oral communication to the Royal Society when he related the phosphorescence "of many bodies" when "cooled between -180°C and -200°C after the stimulation by light". Also Dewar[4] observed that a great increase of phosphorescence "in the cases of organic bodies cooled to the boiling point of hydrogen under light stimulation is very marked, when compared ... (with) the use of liquid air". In these early studies, Dewar, immersed his samples in liquid nitrogen in an open beaker, illuminating the sample from above through the liquid surface.

In 1911, E.L. Nichols[5] published the first review of the earlier work in low temperature luminescence spectroscopy in England, Continental Europe and the United States. Nichols and Merrit in 1911[6] reported the fluorescence and phosphorescence of sixty-three compounds at -190°C and lifetimes between one second and several minutes were observed. Also in 1911, Kowalski[7] and Goldstein[8] initiated a series of reports on cold solutions of aromatic molecules in glicerine-ethanol solutions. During this time several authors explored all types of solvents, including borax solutions, which were called "boric acid phosphors" by Tiede[9]

The study of absorption spectra at differing low temperatures started much later. Although this aspect is not the object of the present study the developments in the study of low temperature absorption

spectra are related to the progress of low temperature luminescence. In 1906 Becquerel[10] observed that crystals of a rare earth element when immersed in liquid air, gave an absorption spectrum which consisted of a large number of very fine bands.

Obreimov and de Haas[11], studying the absorption spectra of azobenzene, potassium dichromate and iodine crystals, observed that at liquid hydrogen temperature the spectra of potassium dichromate and azobenzene exhibited sharp lines. Difficulties in the observation of the absorption spectra of frozen polycrystalline solutions due to problems of light scatter led to the search for transparent glasses. In 1935, Kautsky[12] started to work on ethanol glasses and this work probably guided the development of glasses by Lewis[13] and co-workers who perfected for low temperature luminescence spectroscopy a viscous, highly transparent glass formed from ether, isopentane and ethanol (EPA) in the ratio 5:5:1 by volume. Terenin[14,15] also prepared and used transparent organic glasses.

The Shpol'skii Effect

In 1952, during a study of the fluorescence and phosphorescence of several polycyclic aromatic hydrocarbons, the Russian spectroscopist E.V. Shpol'skii and A.A. Il'ina and L.A. Klimova observed that when coronene was dissolved in n-hexane or n-heptane at liquid air temperature the luminescence spectrum consisted of clearly separated narrow bands[16]. According to Shpol'skii *et al* " an astonishing change in the spectrum was observed when n-hexane and n-heptane were used as solvents. Instead of a small number of bands observed in solutions of paraffin oil, there are a large number of narrow lines which by their width are similar to the lines of atomic spectra in gases". In the

same work Shpol'skii and co-workers[16] also reported an increase in fluoresence intensity in comparison with the emission observed at room temperature. Anthracene, 1,2-benzanthracene, pyrene and 3,4-benzopyrene were also examined: pyrene and 3,4-benzopyrene showed similar distinct narrow band spectra whereas the first two compounds gave broad and diffuse spectra. Shpol'skii and co-workers reported their speculations concerning the possible cause of the appearance of the phenomenon; i.e. the crystalline state of the frozen solution.

In 1954, Bowen and Brocklehurst[17] confirmed the effect, studying coronene at -180°C and also showing that the coronene used by Shpol'skii was contaminated with 1,12 benzoperylene. This was undoubtedly the first analytical application of the effect!!

Later in 1955 the same authors[18,14] reported the continued study of the effect. The fluorescence and phosphorescence spectra of coronene, 1,12-benzoperylene, perylene, triphenylene and 9,10-dichloroanthracene were studied in a series of n-paraffin matrices (n-pentane, n-hexane, n-heptane, n-octane and n-nonane). The influence of the solvent used on the intensity, sharpness, position and splitting of the 0-0 band of coronene was verified and the effects were believed to be due to specific interactions of the solute molecules with the crystal lattice of the solvent and seem to be dependent of the relative sizes of the molecules involved. At the same time Weisman[20] and McClure[21] had reported that the spectra of solid solutions in crystalline solvents appears to be sharper still than in the usual glass-forming solvents. Weisman[20] examined triphenylmethyl in a triphenylamine matrix and McClure[21] used naphthalene in durene and this example suggested to Bowen and Brocklehurst that the Shpol'skii effect was a similar phenomenon.

In 1959 Shpol'skii started a series of reviews[22,24,31,44] where new tentative explanations and models were proposed to explain the phenomenon. It was established that the individual lines of the spectra under discussion corresponded to an electron transition 0-0 and to various vibronic transitions in the molecule. Vibrational analysis was carried out and revealed the occurrence of common frequencies for different molecules[23].

In the review published by Shpol'skii in 1959[22] the first reports of analytical applications appear as the interest grew in the determination of some of the polynuclear aromatic hydrocarbons which are carcinogenic and are present in the environment, in food, effluents, etc.

Chapter II

The Shpol'skii Effect

The similarity between the spectra generated in the Shpol'skii effect and atomic line spectra soon associated a name with this phenomenon - 'quasi-linear'[31] luminescence - as the observed band widths are extremely narrow, of the order of $10cm^{-1}$ and sometimes of the order of $1cm^{-1}$ when temperatures lower than 77K are used and in favourable cases; these compare with half band widths usually observed for the same molecules in glass matrices of the order of $200cm^{-1}$ [24]. Spectra of a similar type can be obtained by the isolation of the molecule under examination at low temperature in matrices of a different type, where the compound in examination forms a mixed substitution crystal as referred to previously, or in inert gases, more frequently noble gases[25]. This general technique is frequently referred to as 'matrix isolation'. It appears, however, that the utility of the phenomenon discovered by Shpol'skii is superior in many respects to that of other methods of obtaining quasi-linear luminescence spectra. In principle it is the most universal, as it uses common solvents and different compounds can produce quasi-linear spectra. On the other hand few stable compounds meet the necessary requirement of the formation of stable substitution solutions and at present it has not been possible to obtain quasi-linear spectra of molecules larger than benzene in stabilised gas matrices[26,27].

One of the basic conditions for the appearance of the Shpol'skii effect is the immobilisation of the molecule in a rigid matrix; under these conditions the masking of the vibrational structure of the electronic transition by the simultaneous rotational excitation of molecules in the gas phase and the thermal fluctuations of the solvent density in the neighbourhood of the molecules of the dissolved compound as found

in liquid solutions are eliminated.

The use of low temperatures normally improves the structure of electronic spectra; in a glassy matrix however the half band width is still usually of the order of 100 - 200cm^{-1}. In order to produce a quasi-linear luminescence or absorption spectrum not only must the frozen solution be polycrystalline but also a suitable geometry relationship between solute and solvent must be present.

The cause of the wide band character of the emission from glassy matrices has been recognized as caused by the disordered structure of glasses: the configuration of the solvent molecules around the molecules of the dissolved substance is different and consequently the pertubations of their energy levels by the solvent are different which leads to a considerable statistical widening of bands. There is thus no short range order in the system. Very recently Personov and co-workers[29,30] have shown that typical quasi-linear fluorescence spectra can be obtained with a glass frozen at 4 K if excitation is effected with a laser at wavelength coincident with the O-O transition of the molecule. This phenomenon is of considerable interest but requires further study and does not change the fact that under normal conditions of excitation and at liquid nitrogen temperature, matrices which form transparent glasses do not produce the Shpol'skii effect. The solvents introduced by Shpol'skii and co-workers[16,22,24] for the study of quasi-linear spectroscopy are n-paraffins of the formula $C_n H_{2n+2}$. These crystallized very easily during freezing when pure. Other solvents of similar behaviour have also been used, such as cycloalkanes as cyclohexane, methylcyclopentane, and isoctane[31,32,33,34].

Basically these are of very weak reactivity, non-polar and only small interaction is expected between the usual type of solute that

produce quasi-linear spectra (polycyclic aromatic hydrocarbons - PAH) and these solvents. The energy of excitation of such a type of solvent molecule is far greater than the excitation energy of the compound dissolved in it. This property has been described as a necessary condition for the production of quasi-linear spectra.

Also the crystalline structure of the matrix must permit the "embedding" of the solute molecules in an 'ordered' manner and in this way eliminate the principal factor concerned in the broadening causing diffuse bands in glassy matrices. The interaction of the molecules with the surrounding matrix is incomparably weaker than the interatomic interaction in these molecules[34]. The best resolution is obtained in one or two members of the n-paraffin family. There are no general rules for the selection of the 'appropriate' solvent; sometimes is possible to establish the relationship established by Bolotnikova[35,36]; these works demonstrated that the best resolution is obtained with polyacenes in those paraffins whose molecular dimensions are similar. Thus, e.g. naphthalene in n-pentane; anthracene in n-heptane; naphthacene in n-nonane.

Compound		n-paraffin	
Naphthalene	7.2 Å	Pentane	7.36 Å
Anthracene	10.0 Å	Heptane	10.0 Å
Naphthacene	12.8 Å	Nonane	12.8 Å

Similar results were obtained with angular hydrocarbons such as chrysene[37,38] picene[39] and benzanthracene[40]. With the series biphenyl-terphenyl-quaterphenyl, Gobov and Bolotnikova[41] showed that this rule is also obeyed. Gobov[42] and Nurmukhametor, Mileshina, Malkes, and Shubina[43] have shown that for stilbene and diphenyl-butadiene and

for various benzoxazoles the rule can also be applied. According to Shpol'skii[44] it is necessary not only to exist the approximate equality of molecular lengths of the compound being studied and the n-paraffin but also a geometric similarity between the ziz-zag arrangement of carbon atoms of the n-paraffins and that of the compounds (Fig. II-1).

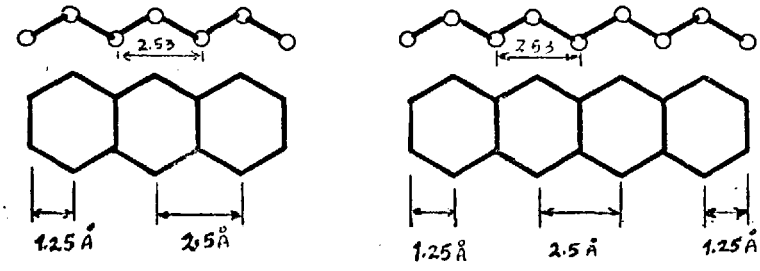


Fig. II-1

For several compounds, however, these rules are not obeyed: e.g. 1,4-distyrylbenzene whose molecule is longer by the length of the styryl group than that of stilbene, shows a better resolved spectrum in n-hexane[45] and not in an n-paraffin with a longer chain.

The manner in which the solute molecules are distributed in the frozen n-paraffin has not yet been explained. The formation of substitution solutions is not in general possible in this case[24,31,44,46].

Shpol'skii[31,44] has invoked the hypothesis that 'clathrate-type' solutions are formed, and in the case of large molecules interblock solutions are present, in which the solute molecules are situated in an orientated manner on the boundary planes of the crystalline matrix blocks[47]. True substitution solutions have been observed by Leach and Lopez-Delgado[48] in the case of benzene and toluene in cyclohexane, which is quite probably due to the similarity in shape and dimensions

of the guest and host molecules[49].

When the solute molecules are not in the appropriate matrix or matrices, wide band or quasi-linear together with wide band or background emission usually appears. The origin of such bands has been studied extensively by Grebenshchikov and Personov, Bolotnikova, Glushkov, Jawonski and Tanin[50-53] who demonstrated that quasi-line spectra are generated by those molecules within the matrix and the wider bands result from those molecules which were segregated out of the matrix during the process of crystallization and can thus interact with each other or other molecules of a second solute[54-56].

The rate of freezing plays inconsequence an important role. During very fast freezing rate of thin layers of solution (150 μm) it is possible to obtain quasi-line spectra with a guest in an unappropriate (unfavourable) matrix as was observed with anthracene in n-decane, pyrene in n-decane, stylbene in n-undecane. In these cases the solute molecules are trapped in the solvent network, whereas during slow freezing diffusion and aggregation are possible[57]. In the case of anthracene in n-decane, using rapid cooling, the spectrum was as sharp as its spectrum in an appropriate solvent (n-heptane), being the 0-0 transition shifted 100cm^{-1} to red but the vibrational fine structure was completely preserved.

The Effect of Concentration on Quasi-linear Spectra

According to most authors, quasi-linear luminescence emission is produced by individual molecules 'isolated' in the solvent matrix low concentrations are therefore expected to favour the state of dispersion and decrease the mutual interaction between the solute

molecules.

The concentration range in which the Shpol'skii effect appears depends on the solute matrix compatibility. Normally there exists one or sometimes up to four, appropriate solvents[54]. In this solvent a further increase in concentration (ca. 10^{-4} - 10^{-3}M) causes diffuse maxima shifted towards the red in relation to the 0-0 transition to appear; with further increase (10^{-2} - 10^{-1}M) it is possible to observe a spectrum similar to the spectrum of the crystalline solute[58]. In some solvents where the Shpol'skii effect is not observed at low concentration, and when only diffuse spectra are observed, by increasing the concentration, quasi-linear emission and absorption spectra may be obtained[54,55,59]. This effect was observed by Ruzevich[60] for azulene in n-hexane and Leach[61] with benzene in n-paraffin at concentrations higher than 1 x 10^{-3}M, whereas in cyclohexane the spectrum remained sharper even at 1 x 10^{-7}M. This led Leach[61] and later Nakhimovskaya[62-63] to suggest that the quasi-linear spectra are due to microcrystalline aggregates[61] or to metastable centres (submicroscopic crystals)[63]. Using a second compound as a 'probe' Klimova et al.[54] shows that no sensitized emission was observed and this reinforced the theory that the quasi-linear spectra result from single isolated molecules.

The effect of the appearance of quasi-linear luminescence at higher concentrations in an inappropriate solvent was explained by Boletnikova and Naumova[64]; these workers suggested that the solute molecules distort the matrix structure with consequent incorporation of the solvent molecules and the production of quasi-linear luminescence.

Klimova, Oglobina and Shpol'skii[65] studied the concentration dependence of the pyrene spectrum in a series of n-paraffins. These

authors divided the solvents in two groups: the solvents of the Group I are solvents which produce, after freezing, an appropriate matrix for the acceptance of the solute molecules; in these solvents regardless of a large range of concentrations and freezing rate the quasi-linear luminescence is obtained.

The Group II solvents are those in which the resolution of the spectrum depends strongly on the solute concentration and on the manner of freezing; the spectrum then consists of broad bands (100 - 250 cm^{-1}) at low concentrations and quasi-linear emission is produced at high concentration.

To explain the effect in high concentrations with the solvents of Group II Klimova and co-workers[65] studied the influence of high concentrations of pyrene (1×10^{-3} - 1×10^{-2} M) on the emission of coronene or perylene in n-pentane. With perylene quasi-linear spectrum appears and the band emission observed when the compound was alone, became weaker in the presence of pyrene. With coronene the spectrum became sharper.

This effect reinforced the theory of deformation of the crystal structure of the matrix. Dupuy, Lochet, and Rousset[66] have suggested that the lack of delayed fluorescence resulting from the triplet-triplet annihilation is an indication of the isolation of the molecules.

Nakhimovskaya, Mishina and Kleshchev[67] reported that under conditions of rapid crystallization of the system, aromatic molecule - normal paraffin, highly supersaturated solid solutions are formed and as a result of supersaturation the resulting single-phase is characterized qualitatively by the Shpol'skii effect. To confirm this hypothesis these workers performed an experiment where solutions of diphenylene oxide were cooled at different rates (slow, 0.03°/sec, and fast, 8°/sec).

The slow cooling produced an absorption spectrum similar to the diphenylene oxide crystal. Fast cooling gave a spectrum similar to the vapour of diphenylene oxide; after ageing isothermally at 163 K for > 240 hours diffusion took place and the spectra were similar to those obtained with slow cooling. These workers then concluded that the results of this experiment agree with the previous explanation of the group nature of the centres responsible for the Shpol'skii effect.

Very recently Kleshchev, Lyamaev, Mishina and Nakhimovskaya[68] studied the effect of higher concentration of some aromatic compounds in the crystalline structure of n-pentane (in the presence of naphthalene; $1 \times 10^{-2} - 5 \times 10^{-1}$M) and n-heptane (in the presence of diphenylene oxide 5×10^{-2}M) both at 77K and using the X-ray diffraction technique. No appreciable change in the crystalline structure of the matrices was observed in comparison with the pure matrices. In the same work an observation using absorption and reflectance spectometry[55] with naphthalene in n-pentane, β-methylnaphthalene in n-hexane and phenanthrene in n-hexane was made. There was observed a relationship between the appearance of bands similar to the crystal absorption (called by the authors 'pseudo-crystalline') and quasi-linear absorption indicating a close connection between the quasi-linear spectra and the spectra having pseudo-crystalline nature. This led the authors to conclude that "quasi-linear spectra are caused by local excitation in segregated phases, while pseudo-crystalline spectra are caused by collective excitation in them"[68].

In a second recent paper Mishina and Nakhimovskaya[69] attempted - by changing the rate of cooling, by using sensitized fluorescence, and by absorption spectrometry - to isolate the pseudo-crystalline spectrum from the quasi-linear spectrum without success. In the opinion of the

authors this confirmed the hypothesis that the two types of spectra are produced by the same centre.

Undoubtedly the confirmation of the observations started by Nakhimovskaya will shed more light on the Shpol'skii effect and probably some of the original concepts of the occurence of this phenomenon will be reformulated.

The Appearance of Multiplet Structure in the Shpol'skii Effect

Multiplet structure was observed in the first quasi-linear spectra[16-19] and a considerable number of studies have been devoted to its interpretation[24,31,44,70-73]. It is now firmly established that one of the reasons for the multiplet structure of such spectra is the presence of several types of radiation centres, due to the solute molecules being situated in different surroundings which cause differences in the disturbances of their electronic levels by the network[24,31,44,72] resulting in a somewhat different energy for the spectral transition. Several hypothesis have been put forward to explain how these differences occur.

a. Crystal Modification

One hypothesis which has been invoked to explain the presence of the multiplet structure has been the existence of several crystal modifications in the solid n-paraffin solution[74]. In the case of benzene in cyclohexane, e.g. when a fast frozen solution is obtained, the two strong components of the multiplet are attributed to the co-existance of crystallites of various polymorphic modifications of the solvent[75]. If the matrix is produced at a rate of freezing when only

one modification is present individual lines rather than multiplets are obtained[76-78]. Changes in the fluorescence spectrum of an n-heptane solution of coronene under repeated coolings of the solution heated to the melting point have been attributed to polymorphic transformations[79]. Mamedov and Laipanov[80] also observed that at temperatures less than 100 K the α and β modifications of n-hexane coexist and consider that this explains the increase in the 'multiplicity' of the phosphorescence spectrum of quinoline.

b. Rotational Isomers

Another hypothesis that has been put forward to explain the differences is one suggested by Shpol'skii[31,44] proposing that in a rapidly solidified matrix besides the stablest forms of n-paraffins molecules, rotational isomers are also present causing distortion in the crystal field and somewhat displacing the energy levels. Although rotational isomers are usual in the gas and liquid state the appearance in the solid state is due, according to Shpol'skii[31,44] to the rate of freezing (one or two minutes) or even at a low rate of freezing (10 minutes) when the rotational isomers might be retained in the crystalline state. This hypothesis has not been tested experimentally.

c. Different Orientations

A longstanding hypothesis by Bowen and Brocklehurst[19] is that the multiplicity is due to the various orientations of the solute molecules in the matrix. Personov and Bykovskaya[81] examined multiplets in the absorption spectra and in the fluorescence spectra of 3,4-benzopyrene in the n-heptane monocrystal and of phthalocyaninein an n-octane monocrystal using polarized light.. They observed that the individual multiplet lines

are differently polarized with respect to the axis of the monocrystal. This confirms that the cause of multiplet structure is due in this case to the various orientations of the solute molecules in the matrix.

The basic hypothesis of different emitting centres has been experimentally confirmed by the studies of the excitation spectra of quasi-linear fluorescence[72,82,83]. Monochromatic excitation of one line of the excitation doublet of coronene caused emission from only one of a corresponding group of lines[84].

A total of 8-20 lines were observed however in the multiplet of certain compounds (3,4-benzopyrene, phthalocyanine) obtained at 20 and 4K[78,85]. It is unlikely that the molecules could orientate themselves in the crystal matrix in so many ways[34].

The multiplet structure can be ascribed to the presence of various centres only in the case where resonance conformity of the multiplet lines of the 0-0 transition lines are observed in the absorption and fluorescence spectra. Personov and co-workers[85] showed that some of the absorption multiplet spectra have more lines than the fluorescence multiplet of the same compound (Mg and **Zn** phthalocyanine). By exciting - with monochromatic radiation from a He - Ne laser-one line without the corresponding emission analogue, no emission was observed at 4K; this was attributed to depopulation by thermal relaxation. On the other hand, the same authors observed that monochromatic excitation of some porphyrin compounds does not reduce the fluorescence emission to one line, although some simplification was observed.

Basically many workers agree that the non-resonance lines of the 0-0 multiplets originate from the coupling of the electron transition of the guest molecule with the local network vibrations (10 - 100 cm^{-1}); the libration of the guest molecule is supposed to have a particular role in this case[46,86,87].

Studying the phosphorescence spectrum of coronene, Grebenshchikov, Butlar and Solodunov[88] observed that there are small differences in the life-time of the two components of the doublet present in the spectrum of this compound.

Effect of Temperature on Quasi-linear Emission

The Shpol'skii effect is extremely sensitive to variation in temperature. Khesina[89] studied the effect of temperature from 77 to 293 K, on the quasi-linear fluorescence spectra of frozen crystalline solutions of some pyrene derivatives (3,4-naphtho - 6,7-benzopyrene, 3'-methyl - 4', 5-ethylene - 3,4,6,7-dibenzopyrene and 3,4,5,6,7-tribenzopyrene) when the lattice of the crystalline solvent was destroyed (at the melting point), the lines of the spectrum diffuse into broad bands; on further increase in temperature up to ambient the nature of the spectrum is unchanged. The background also increased with the increase of temperature. In 1963, Rebane and Kizniakov[90,91] demonstrated that the Shpol'skii effect is produced in a molecule in a crystalline matrix not only by electronic and vibronic transitions accompanied by changes in the vibrational state of the crystalline network (the appearance or disappearance of phonons), but also by no-phonon transitions. Several such transitions, accompanying the 0-0 transition of the solute molecule are presented in figure (II-2)[92]. If the network vibration was harmonic and its frequency independent of the electronic state of the solute molecule, then the energy of all of the zero-phonon transitions would be identical ($E_{00} = E_{11} = E_{nn}$) and only a single spectral line would correspond to this transition, with a frequency $\nu_o = E_{00}/h$, in spite of the superimposition of these no-phonon transitions on the electron transition of the guest[92]. In reality the network vibrators are not

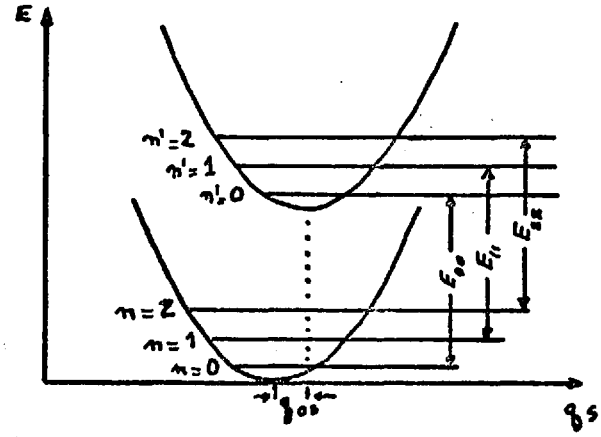


Fig. II - 2 (From Ref. 92)

strictly harmonic and a change in the electronic state of the guest causes - primarily in the close proximity - changes in the position and forces constant of the network oscillators. For this reason the vibration frequencies of these oscillators depend on the electronic state of the guest and $E_{00} \neq E_{11} \neq .... \neq E_{nn}$. In this way a line of a given transition is indeed always the superposition of a set of lines and therefore it is appropriate to use the term quasi-line, first applied by Shpol'skii[31,44]; being a quasi-line a set of no-phonon lines very close to each other. The occupation of the network oscillator level with $n > 0$ decreases with decreasing temperature and with

$$h\nu_s >> kT \qquad \text{II-A}$$

(where ν_s - frequency of the network vibration, k - the Boltzmann constant, h the Planck constant and T - the temperature) we would get only the transitions $n = 0 \rightleftharpoons n' = 0$. However as the network has acoustic vibrations of very low frequency, whose frequencies begin at zero so that even at helium temperature the situation is not solved. Attempts to

determine the internal structure of quasi-lines using the interferometric method have failed since the separation of the individual no-phonon lines was small compared with their width[93]. At 4 K the 0-0 line of 1, 12-benzoperylene produced a band width of 0.9 cm^{-1}. Additional broadening was observed in the vibronic lines, however, and this well correlated with the lifetime of the vibrational states ($\tau \sim 10^{-11}$ sec)[93]. In addition to the no-phonon transitions we find electronic and vibronic transitions in the guest molecules which are accompanied by an exchange with the network, when there is participation of phonons. The number of such transitions falling into the frequency interval of the network vibration (ν_s, $\nu_s + \Delta\nu_s$) is very large[90,91]. As a result these transitions lead to a practically continuous background in the spectrum, the quasi-lines of the no-phonon transitions appearing subsequently over it[93].

As the temperature increases the intensity of the no-phonon transition decreases and the intensity of the phonon background increases rapidly. As a consequence the quasi-lines can only be observed at sufficiently low temperature. The intensity of the 0-0 transition is greater the smaller are the changes in the positions of equilibrium of the network oscillators, i.e. weaker the coupling of the crystal vibrations with the no-phonon transition of the guest molecule. At very low temperature

$$I_{0-0} = e^{-\frac{1}{h\bar{\nu}} \sum_{s=1}^{N} P_s} \qquad \text{II-B}$$

where ΣP_s denotes the so-called Stokes losses of the network oscillators and

$$P_s = m_s \nu_s q_{os}^2/2 \qquad \text{II-C}$$

where m = mass of the s^{th} network oscillator, ν_s its vibration frequency, q_{os} - the change in its equilibrium position marked in Fig. II-2, $\bar{\nu}$-a reliable mean value of the network vibration frequency, which as a crude estimate can be replaced by the Debye frequency[92,94]. From equation II-B we can see that the value of ΣP_s must be small. An estimate for the Shpol'skii matrices is $P \simeq 10^{-2} - 10^{-1}$eV; in comparison $P \simeq 10^{-3}$ eV for the Al_2O_3 crystal with Cr^{+3} as guest (ruby), whereas for the phosphor KCℓ: Tl, which has a wide band spectrum even at 4 K, it is ca. 1.0eV[90,91]. In many works[90,91,95] an analogy has been made between the formation of no-phonon lines during an electron transition of the molecule embedded in the matrix and the Mössbauer effect[96], where the absorption and the emission of a γ-radiation by a nucleus also happens without a phonon, (and also according to Rebane[92] although the Mössbauer line is the narrowest spectral line observed to date the no-phonon gamma line in the Mossbauer effect is in fact a quasi-line). The resonance line of the O-O transition is for this reason called the 'optical analogue of the Mössbauer line' but some theoretical predictions are not confirmed by the temperature-dependent studies of line width[97,98]. With the increase in temperature, decrease in intensity, widening and sometimes shifts of the phonon-free lines has been observed. The mechanism is not entirely clear and attempts have been made to apply the concepts used for doped crystals[99]. For 1,12-benzoperylene in n-hexane the interaction seems to be weak (weak 'electron-phonon' coupling) - the temperature broadening and shift observed is small[98,99]. For perylene in n-octane the coupling is stronger, both shift and broadening are more pronounced. In both cases the shifts were to shorter wavelength (blue shift). The width of the phonon-free line is due, according to the authors, to the simultaneous action of two broadening mechanisms, one which produces a Gaussian line, and is due to the non-ideal nature of the lattice

and independent of temperature, and a Lorentzian line profile which is function of temperature[99-103].

The actual contour results from the convolution of curves of both types and render difficult the interpretation of the data.

Quantitative studies on the effect of temperature on the width and the shifting of quasilines point to two different cases;[99,103] in the first the experimental results can be represented by formulae where an assumption is made that the elastic forces, connecting the molecule with the matrix, change little during the electronic transition. In this case the width is a function of $\sim T^7$ (or T^4) and the shift is $\sim T^4$, at sufficiently low temperature. In the second case the experimental result can be described only on the basis of taking into consideration a strong 'electron-phonon' coupling[99,104].

The widening that occurs between 77K and the melting point of the crystal comprises more than 85% of the total and also the quasilines can exhibit either red or blue shift as the temperature increases[104]. According to Sapoznikhov[104] the thermal expansion of the lattice would be quite large and would, therefore, have a significant effect on the shift of phonon-free lines.

The Effect of Pressure on Quasilinear Emission

One possibility to study the expansion of the lattice is by the observation of the effect of pressure on the spectrum[34,105]. Hein and Offen[105] studied the effect of pressure on the spectrum of 1,12-benzoperylene in n-heptane and coronene in n-heptane. Red shifts were observed of the orders of 8.3 cm^{-1}/Kbar for 1,12 benzoperylene and 5 cm^{-1}/Kbar; with chromium ions in Al_2O_3 the ratio is 0.3 cm^{-1}/Kbar

showing the 'softness' of this n-paraffin matrix[106]. High pressure broadens the vibronic bands and increases the background intensity. The 0-0 band becomes more pronounced under pressure[105].

Chapter III

Instrumental Techniques and Qualitative Applications

Previous Work

Monochromators

One of the necessary conditions for the successful observation of the Shpol'skii effect is the use of a monochromator of high resolution. To be analytically useful the instrument must also have high light gathering power. When spectra are recorded with an instrument of low resolving power, little difference in the width of the lines is observed in glassy and polycrystaline matrices, and also loss of the fine structure is observed. This results from the fact that with a low resolution monochromator the band-widths observed are merely images of the spectrometer entrance slit.

The first observation of the phenomenon by Shpol'skii[16] was made using a glass prism spectrograph with a reciprocal dispersion of 2.5 nm mm^{-1} in the region of 400 nm.

Bowen and Brocklehurst[17-19] observed the effect with a Hilger constant deviation spectrometer with a glass prism. In the blue region the wavelength accuracy was $\pm$ 0.1 nm or better and in the yellow region of $\pm$ 0.5 nm.

Spectrographs were, and largely still are in use by Russian workers. In his first review concerning the phenomenon in 1959, Shpol'skii[22] refers to two spectrographs. One had a glass prism, with a reciprocal linear dispersion of 2.7 nm. mm^{-1} at 400 nm, and other had a plane diffraction grating which produced a reciprocal linear dispersion of 0.49 nm. mm^{-1}. Similar instrumentation was used by Il'ina and Personov for qualitative analysis work[107].

Muel and Lacroix,[108] and Lavalette, Muel *et al*[109] employed one monochromator with two flint prisms. This had 'great luminosity and great

purity of isolated light'. Shpol'skii and Personov[110] refer in 1962 to the use of a spectrograph with a reciprocal dispersion of 0.3 nm mm^{-1} in a review concerning the application of the method for analytical purposes. Jäger and Lugrova[111,112] have been applying the effect to analysis using a glass prism spectrographs(KSA-1) with a linear dispersion of 0.55 nm mm^{-1} at 400.0 nm. Most Russian workers use the ISP-51, a spectrograph. This has three glass prisms and produces a dispersion of 0.65 nm mm^{-1} at 400.0 nm[113-116,118,119,122-126].

Also the use of diffraction spectrograph[120] is frequently reported (DFS-12); no specification of the performance of this system appears in the published work, however. A high transmission spectrograph has also been used[117,126] (ISP-73) this is a dual glass prism spectrograph with a reciprocal dispersion of 2.0 nm. mm^{-1} at 410 nm and a relative aperture of 1:4.

Eichhoff and Köhler[121] used a spectrograph with three glass prisms; no other details were given, however.

Sources of Excitation

A mercury vapour discharge lamp has been used in most reports[113-123,126] by Russians workers. Typical mercury lamps quoted are of the type PRK, DRSh and SVDSh. The PRK type of lamp is of high pressure (of the order of 1 atmosphere) and has a quartz envelope: the PRK-2[113,123] and PRK-4[107,127] are some of the types in use: the PRK-2 has a power rating of 375 watts and the PRK-4, 220 watts[128]. The DRSh type of lamp is also employed; this is a spherical ultra-high pressure mercury vapour lamp and is used in combination with a suitable lens. The DRSh-250 is one

of the most frequently used and has a power rating of 250 watts[116,118, 119,124,129-130]. Another type of mercury lamp is the SVDSh-250. The SVD is a mercury vapour lamp which has a brightness less than the DRSh lamp but exhibits a large fraction of its energy in the short wavelength ultra-violet region. The suffix Sh refers to the type of assembly - 'sharovaya'-spherical[128] and probably has a power rating of 250 watts.

Jäger and Lugrová[111] also employed a Phillips mercury lamp (57202E / 70) with 125 watts of power. Eichhoff and Köhler[121] used a high pressure mercury lamp (Phillips SP 500) probably 500 watts.

In contrast to the mercury lamps, Xenon sources have not so frequently been used. Fedoseeva and Khesina[124] employed a Xenon lamp (DKSSh-1000) probably 1000 watts of power rating. Lavalette et al[109] used an Osram 900 watts Xenon lamp.

Selection of Wavelength of Excitation

Optical filters are most commonly used for selection of the wavelength of the radiation used for excitation. Russian workers have used filters of the type UFS. The UFS-1[114] is used to isolate the wavelength range from 250 to 400 nm[128]. Actually the band is wider and some radiation also is transmitted at 240 nm and at 420 nm. This filter has been reported to have the disadvantage of only low life stability. The UFS-2 filter[122] is used to isolate the 270-380 nm range and absorbs almost all visible radiation[128]. The UFS-3[107,116-7] is more frequently used and behaves like Wood's glass, isolating the 320-390 nm region[128]. Eichhoff and Köhler[121] employed a combination of two glass filters, the UG2 and the UG11 (Jenaer Glasswork, Mainz, Germany); no other information is given, however.

The use of a monochromator to select the excitation wavelength radiation has been less frequently reported; Fedoseeva and Khesina[124] used an excitation monochromator (a ZMR-3) but no further description was given. Lavalette[109] employed a dual quartz prism monochromator for excitation similar to that employed for recording the emission spectra.

Sample Cell

Several types of cells have been used to study the Shpol'skii effect; the degree of sophistication of these cells is usually a function of the temperature or the coolant employed. For analytical purposes liquid nitrogen is normally used, although there has been a report of the use of liquid air for this purpose[121].

More details concerning sample cells is given in Chapter VII.

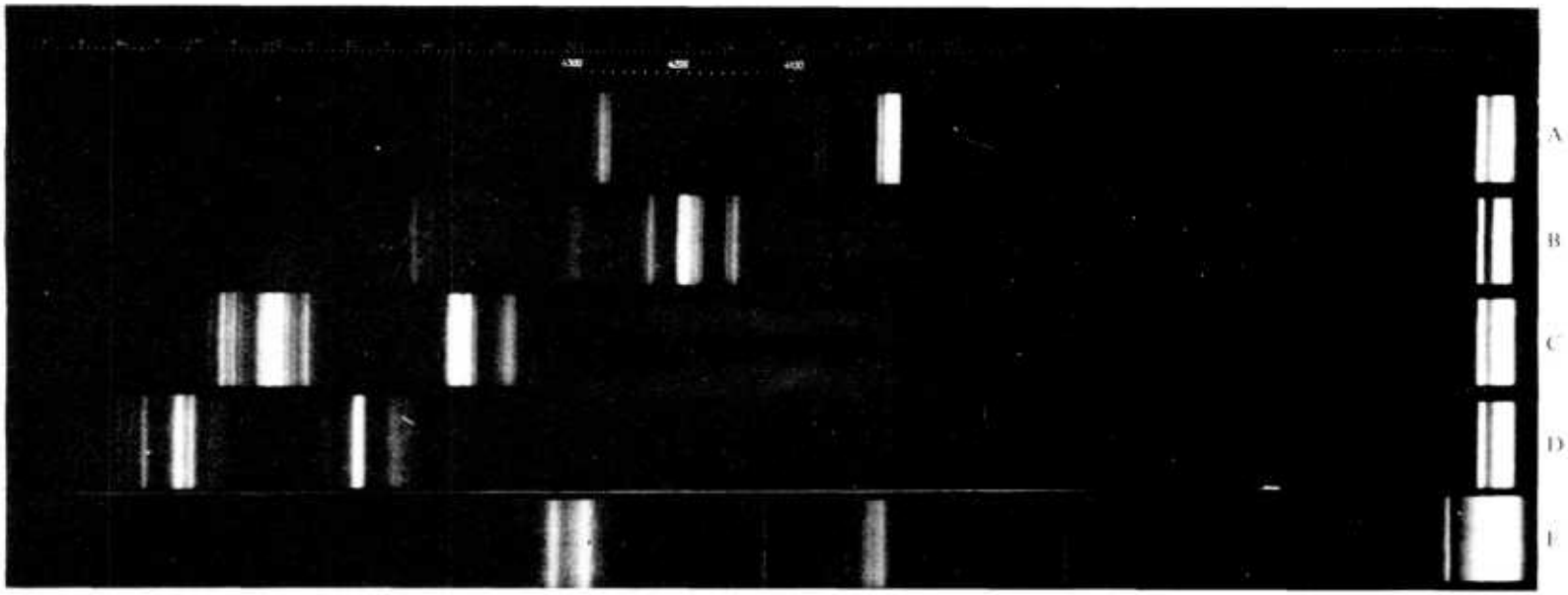
Detection of Luminescence Radiation

Most of the earlier studies of quasi-linear luminescence spectrometer were conducted with the photographic plate system of detection[112] (Fig. III-1). Most recent Russian papers still deal with both photometric and photographic plate methods.

A FEU-17 photomultiplier has been used in some of the Russian work[116,118]. This photomultiplier has a photocathode of antimony-cesium but no other specification has been revealed from the published papers. Muel and Lacroix[108] and Lavalette[109] used the phothometric technique with an EMI 6685B photomultiplier as the detector; this photomultiplier has been superseded by the EMI-9502B, which has an S-11 response[131].

Eichhoff and Köhler[121] used a spectrograph fitted with an RCA

from Ref. 112



Quasilinear fluorescence spectra of pure substances A – benzo(a)pyrene, B – benzo(ghi)perylene, C – anthanthrene, D – perylene, E – benzo(k)fluoranthene.

Fig. III-1

1P21 photomultiplier (S-4 spectral response)[132] to provide a direct reading photometric system.

Applications of the Shpol'skii Effect in Qualitative Analysis

A very large volume of work has been concerned with the use of molecular luminescence spectrometry for the identification and determination of traces of organic compounds in mixtures[135-141]. Spectrofluorimetric procedures in which careful choice of wavelength of the excitation and emission have thus been used to distinguish between individual components.

The properties of 'quasi-linear' spectra which facilitate the identification of compounds that produce the Shpol'skii effect are basically the available specificity and the selectivity[110]. The specificity is high because the 'quasi-linear' spectra represent strictly the individual properties of the molecule being studied. This is even true for isomers -- as for example 3,4-benzopyrene and 1,2-benzopyrene whose quasi-linear spectra are quite different and cannot possibly be confused (Figures III-2). The position and number of substituents on a nucleus also gives rise to a considerable difference in the spectral characteristics of the quasi-linear luminescence emission. Thus, e.g. in mono-methyl derivatives of 1,2-benzanthracene[142] and methyl derivatives of 3,4-benzopyrene[143] a specfic influence is observed, sometimes in the form of a wavelength shift or sometimes in the form of changes in the distribution of the intensities of the main lines of spectrum. The selectivity is also good owing to the characteristics of the spectra and the sharpness of the lines. It is thus possible to establish the presence of a given compound in a complex mixture. By the use of selective excitation and with the help of the phosphorescence

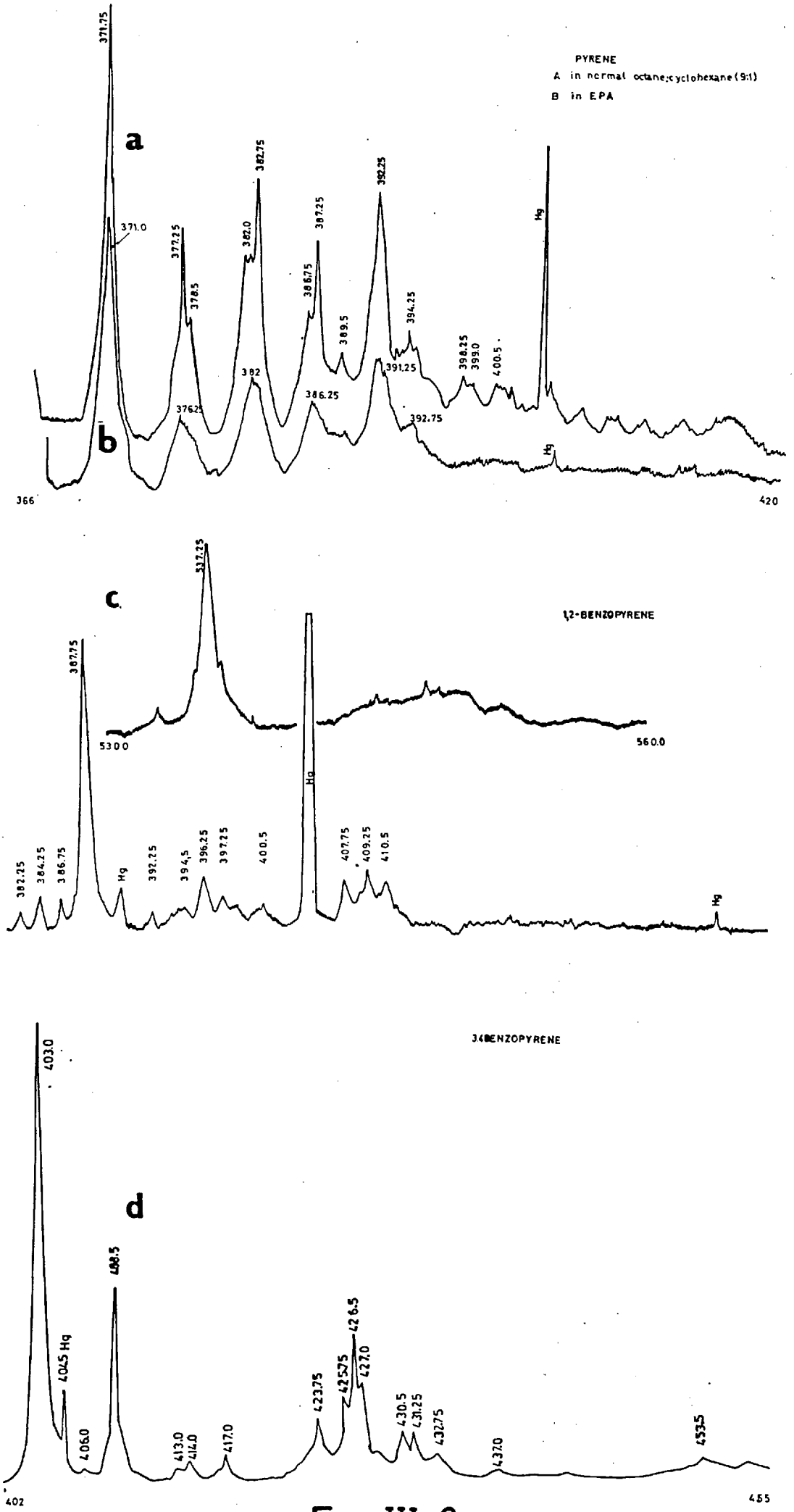

PYRENE
A in normal octane:cyclohexane (9:1)
B in EPA
a
b
c
d
1,2-BENZOPYRENE
3,4BENZOPYRENE
Hg
366
420
530.0
560.0
402
455

Fig. III-2

spectrum when present, the selectivity can be improved.

The use of quasi-linear spectra for qualitative analytical purposes was first reported by Dikun and co-workers[144-6] in 1959; they recommended the use of the method for the positive identification of 3,4-benzopyrene in samples of very diverse origin, e.g. polluted air, tobacco smoke, shale tar, etc. using n-hexane or n-octane as solvent at 77 K. Il'ina and Personov[22,147] have applied the technique to the detection of 1,12-benzoperylene in extracts of Jurassic and Cretaceous sedimentary rocks and perylene in tertiary sediments.

Dikun[148] identified pyrene, 1,2-benzopyrene, 3,4-benzopyrene, perylene, 1,12-benzoperylene and 1,2,7,8-dibenzanthracene in smoked fish and polluted air by using the quasi-linear emission in an n-hexane solvent at 77 K, also using the same technique Dikun[148,149] detected 2,3-O-phenylenepyrene, 1,2,4,5-dibenzopyrene and 3,4,9,10-dibenzopyrene in polluted air. Gurov and Novikov[150] identified anthracene, pyrene, 3,4-benzopyrene, 1,12-benzoperylene, perylene and coronene in soil and snow samples using the quasi-linear luminescence spectra obtained at 77 K in an n-hexane solvent.

Parker and Hatchard[151] have applied the technique using n-octane: cyclohexane as solvent to an investigation of an unusual photo-reaction of 3,4-benzopyrene in solutions containing polymer: three closely related photochemical reaction products were readily distinguished from 3,4-benzopyrene, although the four compounds were indistinguishable from one another at room temperature.

Florovskaya, Teplitskaya, Pikovskii and Ovchinnikova[152-3] identified 1,12-benzoperylene, 3,4-benzopyrene and perylene in rocks and minerals in hydrothermal deposits and in regions where oil and gas deposits were found. Arabidze[154] used the technique of quasi-linear luminescence spectrometry for determining and monitoring the purity of coronene -

usually contaminated with 1,12-benzoperylene - and the purity of pentacene. Lavalette, Muel, Hubert-Habart, René and Latarjet[109] applied selective excitation together with quasi-linear luminescence to study the spectral characteristics of seven polycyclic aromatic hydrocarbons (1,2-benzanthracene, 1,2,5,6-dibenzanthracene, pyrene, 3,4-benzopyrene, 3,4,8,9-dibenzopyrene and 3,4,9,10-dibenzopyrene and 3,4-benzofluoranthene) both alone and in admixture. The conditions of detection were dictated by the type of monochromators used for the excitation side and for the emission side.

Florovskaya, Vdvokii, Teplitskaya and Zezin[155] applied the Shpol'skii effect for the identification of PAH compounds in carbonaceous chondrites (meteorites) and in rocks and in minerals. Anthracene, 3,4-benzopyrene, 1,12-benzoperylene and coronene were identified; these are the predominant polycyclic aromatic hydrocarbons formed in high temperature formation of rocks and in the meteorites analysed.

Note on nomenclature

Throughout this thesis the nomenclature used for the polycyclic aromatic hydrocarbons is the same as that of the book 'Polycyclic Hydrocarbons' by E. Clar[165] which is used in most of the papers - especially the Russian - consulted. This nomenclature is however pre I.U.P.A.C. - rules of 1957.[165] The table below shows some of the names in the two nomenclatures and some of the structures of the compounds studied can be seen in Fig. III-3.

Table III-1

	Reference (165)	I.U.P.A.C.
a.	1,2-Benzanthracene	Benzo(a)anthracene
b.	3,4-Benzofluoranthene	Benzo(b)fluoranthene

c.	1,2-Benzopyrene	Benzo(e)pyrene
d.	3,4-Benzopyrene	Benzo(a)pyrene
e.	1,12-Benzoperylene	Benzo(ghi)perylene
f.	2,3-O-Phenylenepyrene	Indeno(1,2,3-cd)pyrene
g.	1,2,5,6-Dibenzanthracene	Dibenzo(ah)anthracene
h.	3,4,8,9-Dibenzopyrene	Dibenzo(ah)pyrene
i.	3,4,9,10-Dibenzopyrene	Dibenzo(ai)pyrene
j.	1,2-Benzonaphtho-(2',3':4,5) pyrene	Benzo[a]naphtho [8,1,2-cde] naphthacene

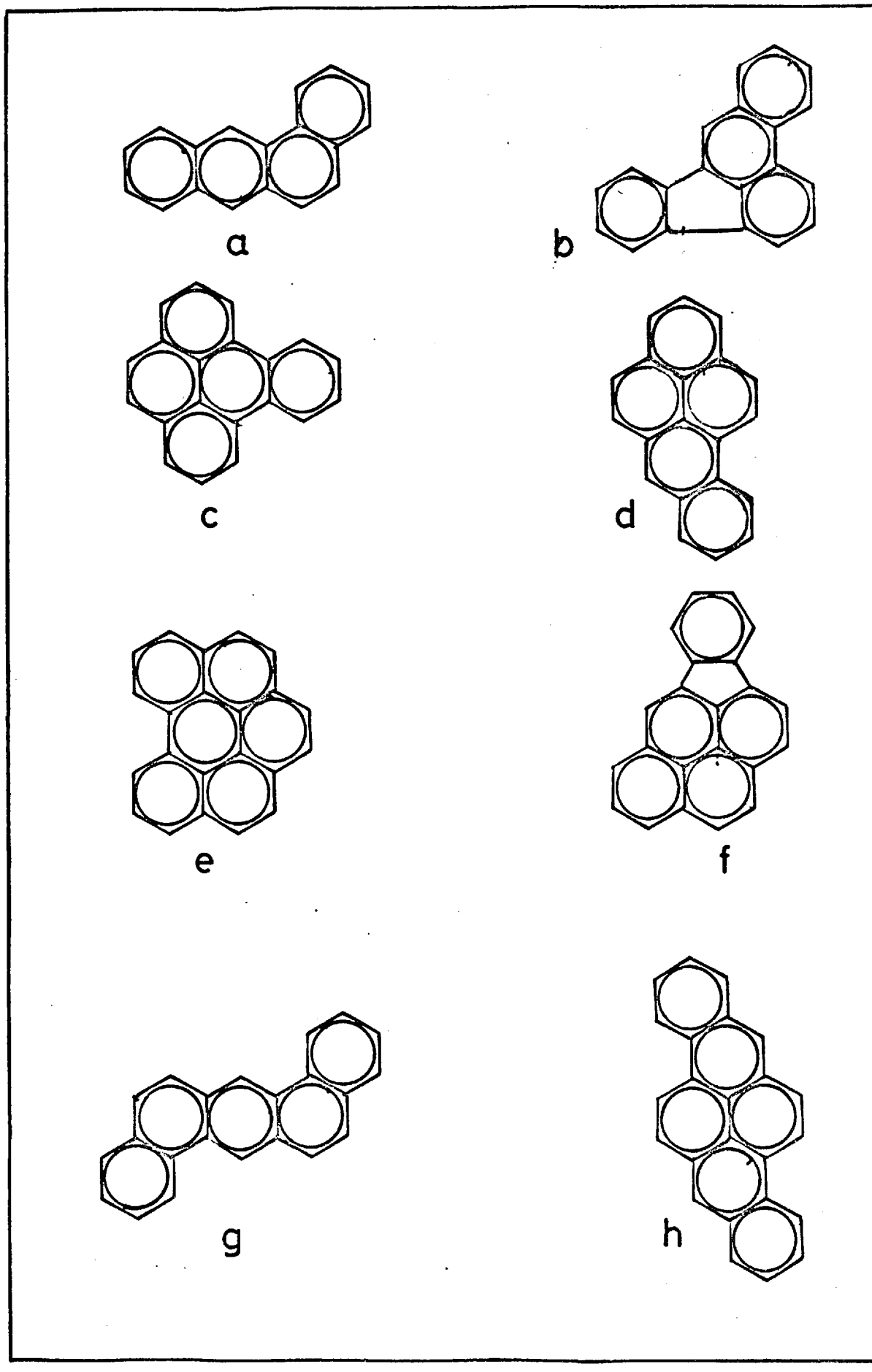


Fig. III-3

Chapter IV

Qualitative Analysis Experiments

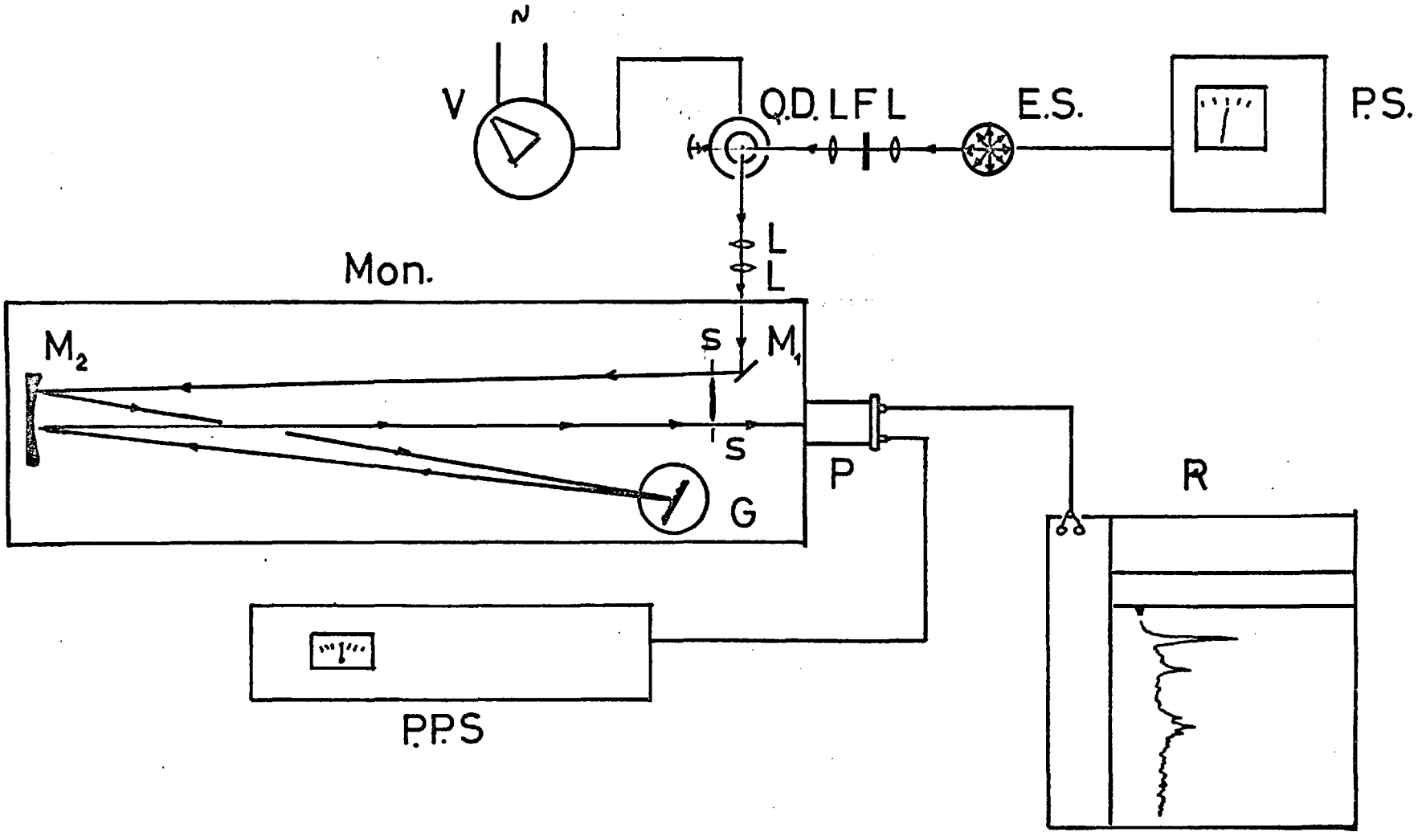
In order to examine and evaluate the characteristics of quasi-linear luminescence and study the qualitative aspects of the phenomenon a simple spectrometer system was assembled. The system consisted of an excitation source, a sample holder, a monochromator and a read out system. Also an Aminco (American Instrument Co.) spectrofluorimeter was used for comparison of high resolution spectra with those obtained at low resolution and for determination of the excitation spectrum. The excitation source employed for the quasi-linear fluorescence studies was a medium pressure mercury vapour discharge lamp (Wotan Hg/3) with 50 watts of nominal power which was driven by a homemade a.c. power supply.

The radiation from this source was focussed into a light tight sample cell compartment by using two silica lenses of 45 mm diameter and 75 and 50 mm focal length. Interferences filters, (Baird-Atomic Ltd.) 50 mm x 50 mm and of narrow spectral band-width (half band-width of 14 mm at 250 mm and 30 nm at 300 nm) were employed to select the excitation wavelength. The filter was inserted between the source and sample cell compartment. The sample tubes employed were constructed from silica tubing (Spectrosil, Jencons Ltd., Hemel, Hempstead) sealed at one end and 200 mm in length, 3 mm i.d. and 0.6 and 1.0 mm wall thickness. These sample tubes were used with the silica Dewar flask from the low temperature accessory of the Aminco low resolution spectrofluorimeter. Approximately 0.3 - 0.5 ml of solution was required for these sample tubes. The silica tube with the sample was plunged into the liquid nitrogen contained in the Dewar flask so as to achieve rapid freezing. The flask was then placed in the sample cell compartment.

The scanning monochromator used was a grating monochromator (Optica Model CF-4, Optica, Italy) with a reciprocal linear dispersion of

1.6 nm mm^{-1}. This monochromator uses a Littrow-type assembly and a plane grating as a dispersing element. The grating is ruled with 600 lines. mm^{-1} and covers the range of 185-1000 nm.[156] The entrance and exit slits are coupled and have a free height of 20 mm and are calibrated for width in steps of 0.02 mm. The stray light according to the manufacturers specification is 0.2%. The wavelength is strictly linear and is calibrated in steps of 0.5 nm. There is no information concerning the optical aperture: based in the focal length and the size of the optics employed an estimate of f/10 can be made. A clock motor, driven by the mains was used as a scanning device. A scan velocity of 6nm min^{-1} was used in all experiments.

Owing to the physical construction of the monochromator the sample compartment was located in such a manner that the excitation optical path was at 90^{o} to the emission optical axis. (Fig. IV-1). Due to this incident light was slightly defocussed at the surface of the frozen sample; this defocussing of the incident light was found to give rise to more reproducible signal intensities than when the source radiation was brought to a focus at the sample surface. Luminescence from the sample cell was focussed on the entrance slit of the monochromator by using a compositebiconvex silica lens (40 mm diameter and 35 mm focal length). An end-window photomultiplier tube (EMI 9601B) was used; this is an S-11 type photomultiplier equipped with a blown window of Corning 9741 glass to extend the useful spectral range to ca. 185.0 nm[157]. The luminescence signal was recorded directly at a potentiometric chart recorder. (Servoscribe Model RE 511.20) using a load resistor of 24 KΩ. and a damping capacitor of 2 μF. In some experiments a microameter (RCA model WV-84C) was employed for signal amplification and registration.



V-Variac. M_1, M_2-Mirrors. Q.D-Quartz Dewar. L.Lens. F Filter. E.S.Excitation Source P.S.Power Supply. Mon.Monochromator. s.slits. G..Grating. P. Photomultiplier. R.Recorder. P.P.S-Photomultiplier Power Supply.

Fig. IV-1

Reagents

The alkane solvents used were n-pentane, n-hexane, n-octane and n-decane. In the preliminary experiments these solvents were used without further purification and were laboratory reagent grade (BDH). Cyclohexane (BDH, laboratory reagent-grade) was purified by passage through silica-gel (60 - 120 mesh), which had been activated overnight at 120 to 130°C.

Also some luminescence observations were also made in a glass forming matrix; EPA was used as a solvent (diethyl ether-isopentane-ethanol 5:5:2) and was prepared with ether that had been dried with sodium wire and isopentane, dried with sodium wire and percolated through silica-gel. Ethanol was treated with potassium hydroxide and distilled. The silica-gel was recovered by treatment with a dilute solution of potassium permanganate in dilute sulfuric acid solution overnight; the manganese salts were then treated with a solution of oxalic acid, washing until neutral and dried, later this procedure was also used with new batches of silica-gel that showed a faint yellow colour.

Materials

Samples of pure polynuclear aromatic hydrocarbons compounds were kindly donated by the Tobacco, Co.Southampton and Shell Research Ltd., Thornton Research Centre, Chester. 4,9-di-tert-butyl-pyrene and 3,5,8,10-tetra-isopropylpyrene were kindly provided by Professor Arne Berg, University of Aarhus, Denmark.

Procedure

Low resolution excitation and emission spectra were recorded in EPA solvent at 77K with the Aminco spectrofluorimeter with the low temperature cell attachment already described or at room temperature with a silica cuvette (10 x 10 x 30 mm) using solutions in cyclohexane. For examination of the quasi-linear luminescence spectra stock solutions of the polycyclic aromatic compounds were prepared in cyclohexane; these solutions were diluted to a concentration of 20 or 2 $\mu g\ ml^{-1}$ with the appropriate n-alkane and cyclohexane so that the final solutions examined contained 10 per cent V/V of cyclohexane. These solutions were transferred into the silica tubes which were then introduced into the Dewar flask containing liquid nitrogen. Rapid freezing of the sample solutions was then obtained. After the initial vigorous boiling action of the liquid nitrogen had subsided, the luminescence spectrum is scanned (using a pre-selected filter for excitation based on the excitation spectrum produced at low resolution).

Results

Cyclohexane is a generally suitable solvent for polycyclic aromatic hydrocarbons and its presence at a concentration of 10 per cent V/V in the n-alkane solvents used for low temperature luminescence work has been demonstrated not to disturb appreciably the spectra obtained,[108,151,158] Muel and Lacroix[108] observed that the addition of 10% of n-octane in a mixture with benzene and cyclohexane was sufficient to produce the quasi-linear spectrum of 3,4-benzopyrene similar to the spectrum obtained by previous evaporation, until dry, of a solvent mixture

and later solubilization with n-octane. As only small amounts of some polycyclic aromatic hydrocarbons (PAH) compounds were available to us it was therefore decided to use cyclohexane for their dissolution mixtures (90 + 10) for dilution of the stock PAH solutions for examination of their luminescence spectra.

An examination of earlier work[23,108,120,124,125] revealed that n-octane had proved to be suitable as a solvent for study of the Shpol'skii effect for some PAH compounds. Initially, therefore, the low temperature luminescence emission spectra of twenty two compounds were recorded in n-octane: cyclohexane (90:10 V/V). Excitation wavelengths were selected from the corresponding low temperature excitation spectra (in EPA) that were obtained using the low resolution double monochromator spectrofluorimeter.

The wavelength was chosen to correspond to that of the most intense low-wavelength excitation peak in order to minimise observed scattered light during the study of the emission spectrum. Of the compounds examined in n-octane-cyclohexane solvent at 77 K, triphenylene, chrysene, perylene, pyrene, 3,4,8,10-tetra-isopropylpyrene, 3,4-benzopyrene, 1,2-benzopyrene, 1,2,3,4-dibenzopyrene, 1,2,4,5-dibenzopyrene, 3,4,9,10-dibenzopyrene, 1,2-benzanthracene and 1,2,5,6-dibenzanthracene exhibit quasi-linear luminescence in which emission peak half-widths of 0.5 nm or less were recorded.

The spectra observed for 2 or 20 μg ml^{-1} solutions of these compounds in n-octane-cyclohexane at 77 are shown in Fig. IV-2 to Fig. IV-6. The compounds 3,4,8,9-dibenzopyrene, (Fig. IV-2C), 2,3-O-phenylene pyrene [indeno (1,2,3-c,d) pyrene]; benzo (1, 2) naphtho (2',3': 4,5) pyrene; [benzo [a] naphtho [8,1,2-cde: naphthacene] (Fig. IV-3), 3-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene,

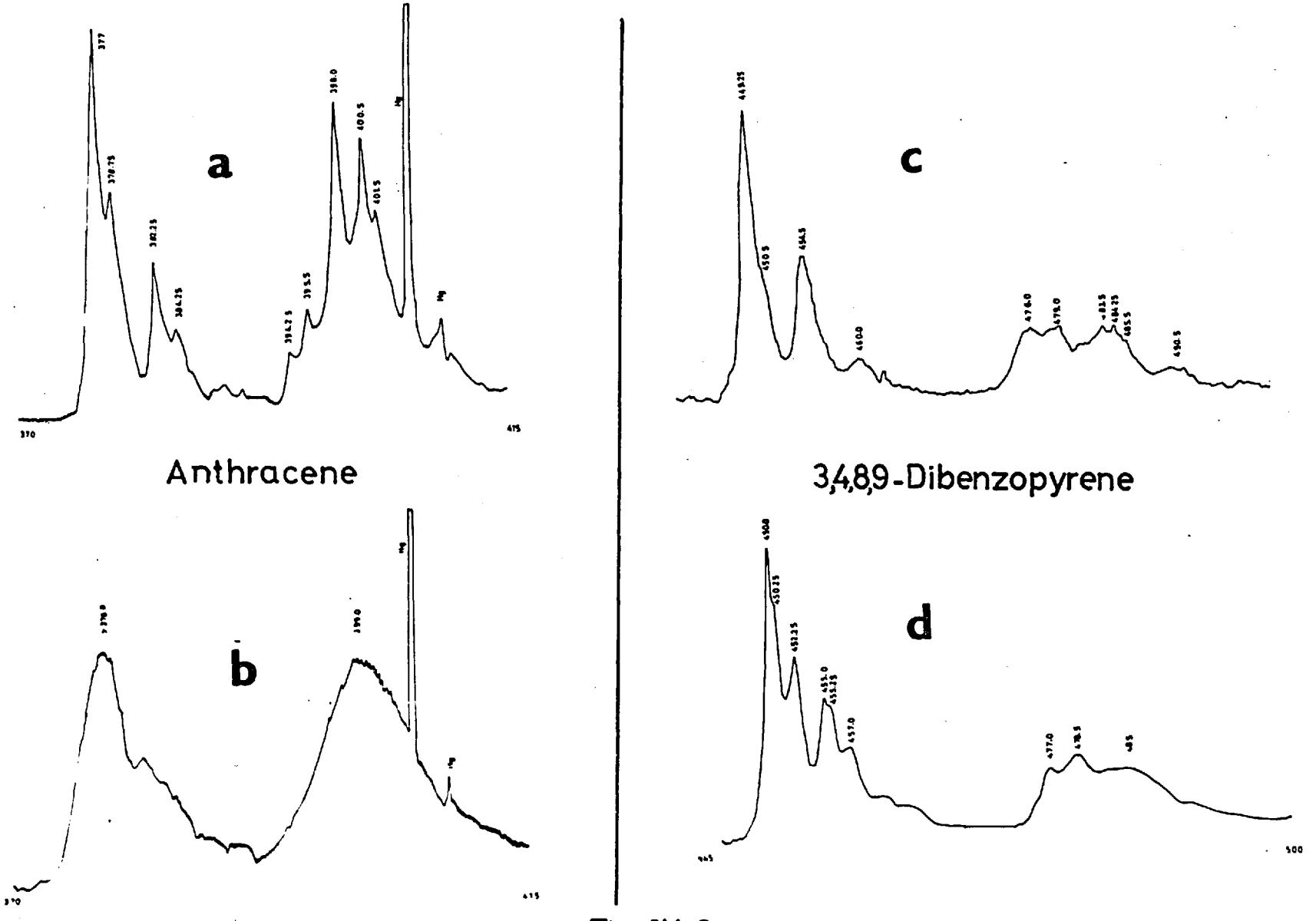


Fig. IV-2

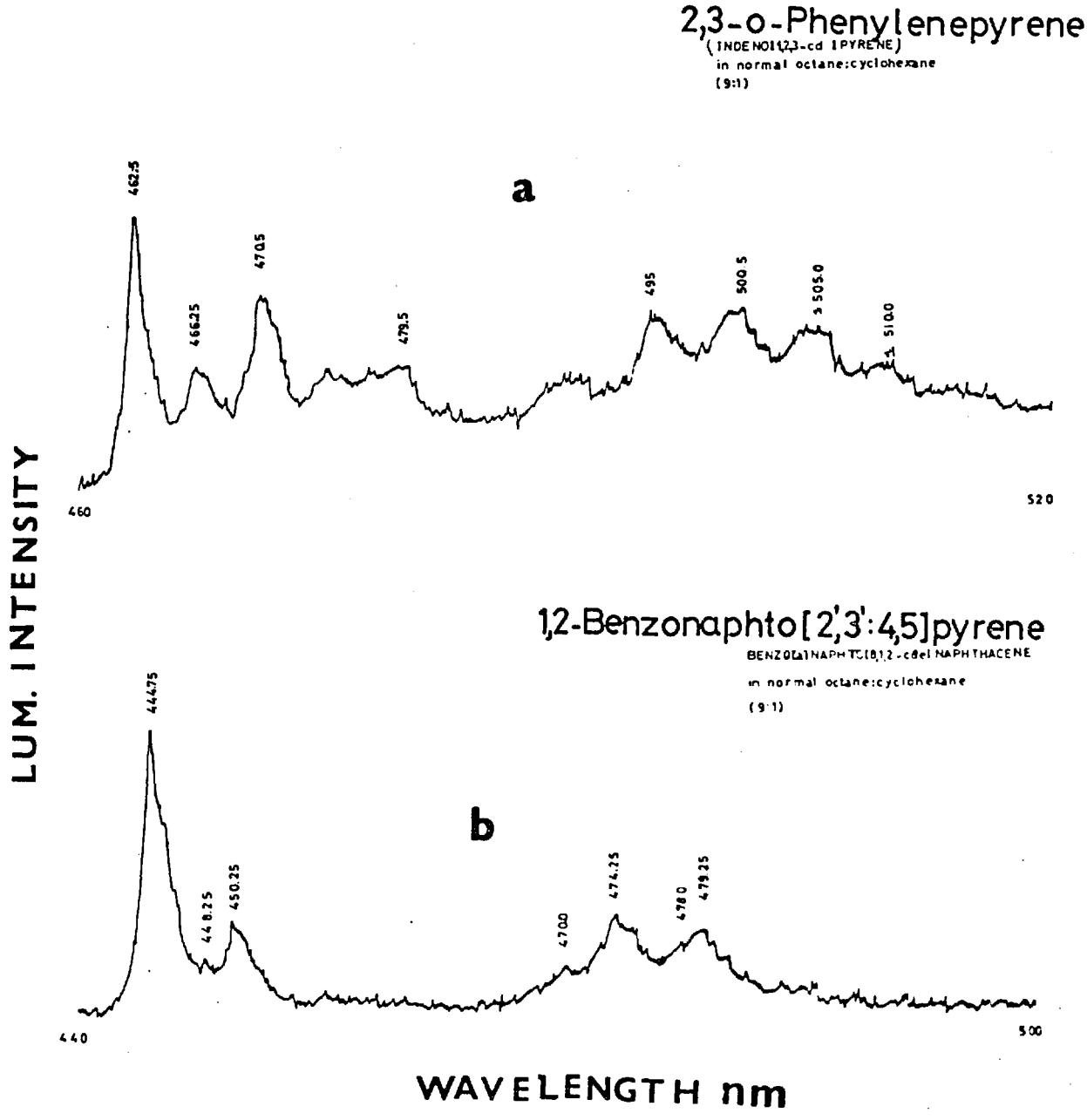

2,3-o-Phenylenepyrene
(INDENO[1,2,3-cd]PYRENE)
in normal octane:cyclohexane
(9:1)
a
462.5
466.25
470.5
479.5
495
500.5
≥ 505.0
≤ 510.0
460
520
1,2-Benzonaphto[2',3':4,5]pyrene
BENZO[a]NAPHTO[8,1,2-cde]NAPHTHACENE
in normal octane:cyclohexane
(9:1)
b
444.75
448.25
450.25
470.0
474.25
478.0
479.25
440
500
LUM. INTENSITY
WAVELENGTH nm

Fig. IV-3

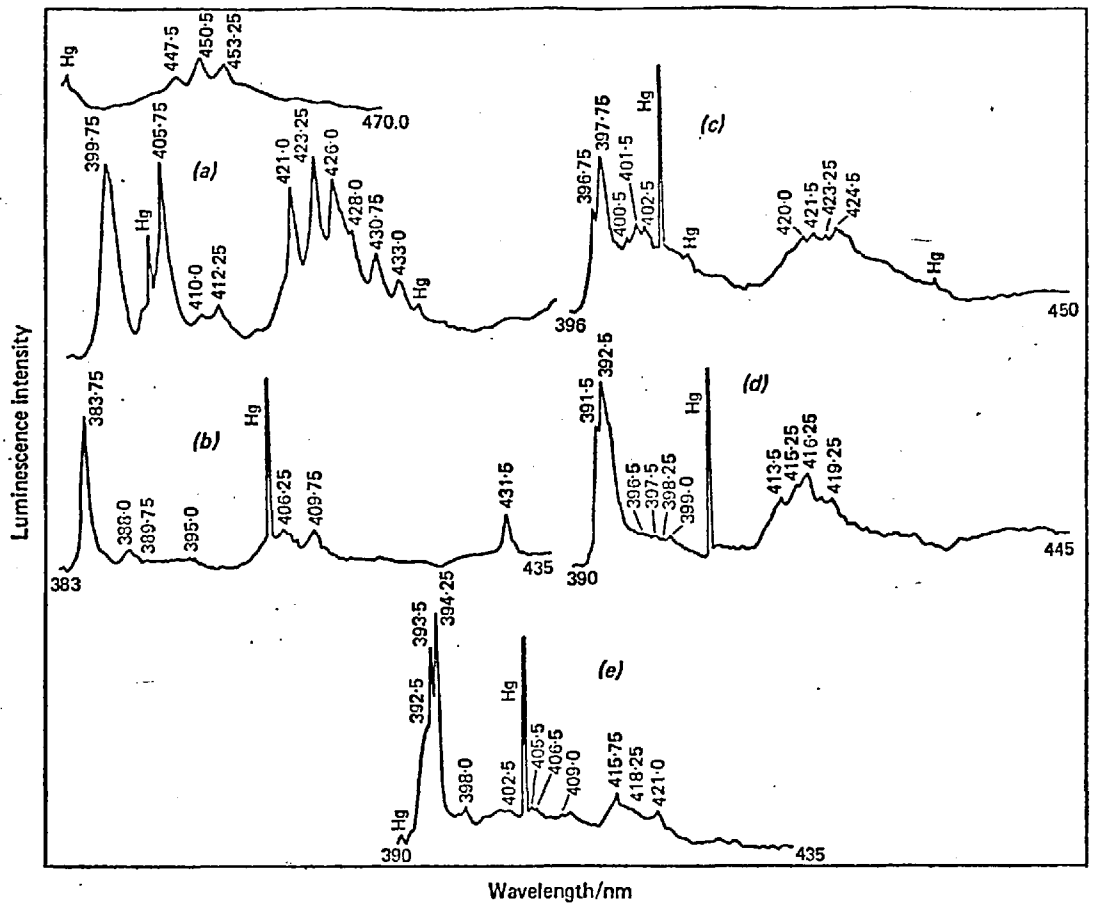


Fig. IV-4 Emission spectra at 77 K of (*a*) 9,10-dimethylanthracene in n-hexane - cyclohexane; and (*b*) 1,2-benzanthracene, (*c*) 9,10-dimethyl-1,2-benzanthracene, (*d*) 3-methylcholanthrene and (*e*) 1,2,5,6-dibenzanthracene in n-octane - cyclohexane. From Ref. 191.

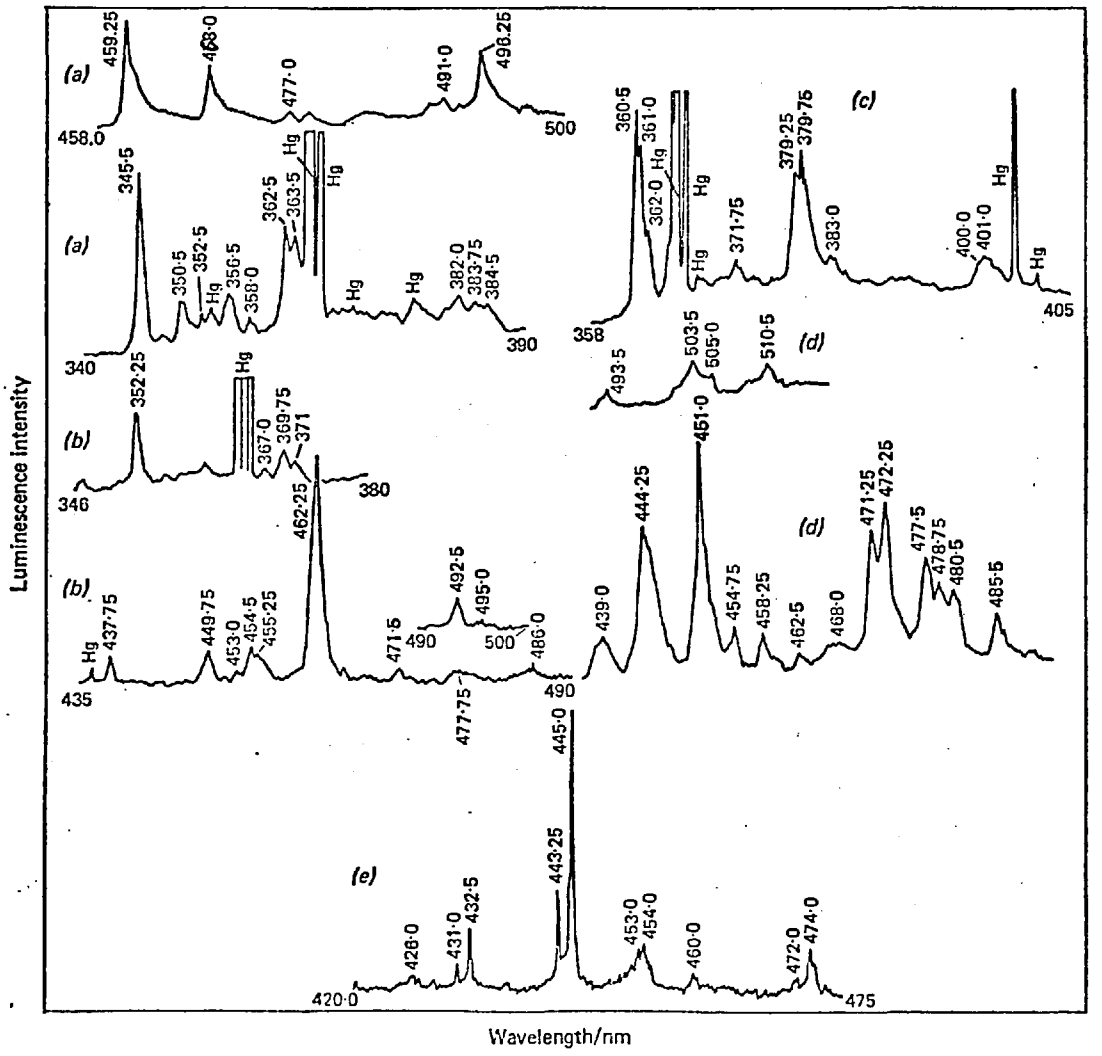


Fig. IV-5 Emission spectra at 77 K of (*a*) phenanthrene in n-hexane - cyclohexane; (*b*) triphenylene in n-octane - cyclohexane; (*c*) chrysene in n-octane - cyclohexane; (*d*) perylene in n-octane - cyclohexane; and (*e*) coronene in n-hexane. From Ref. 191.

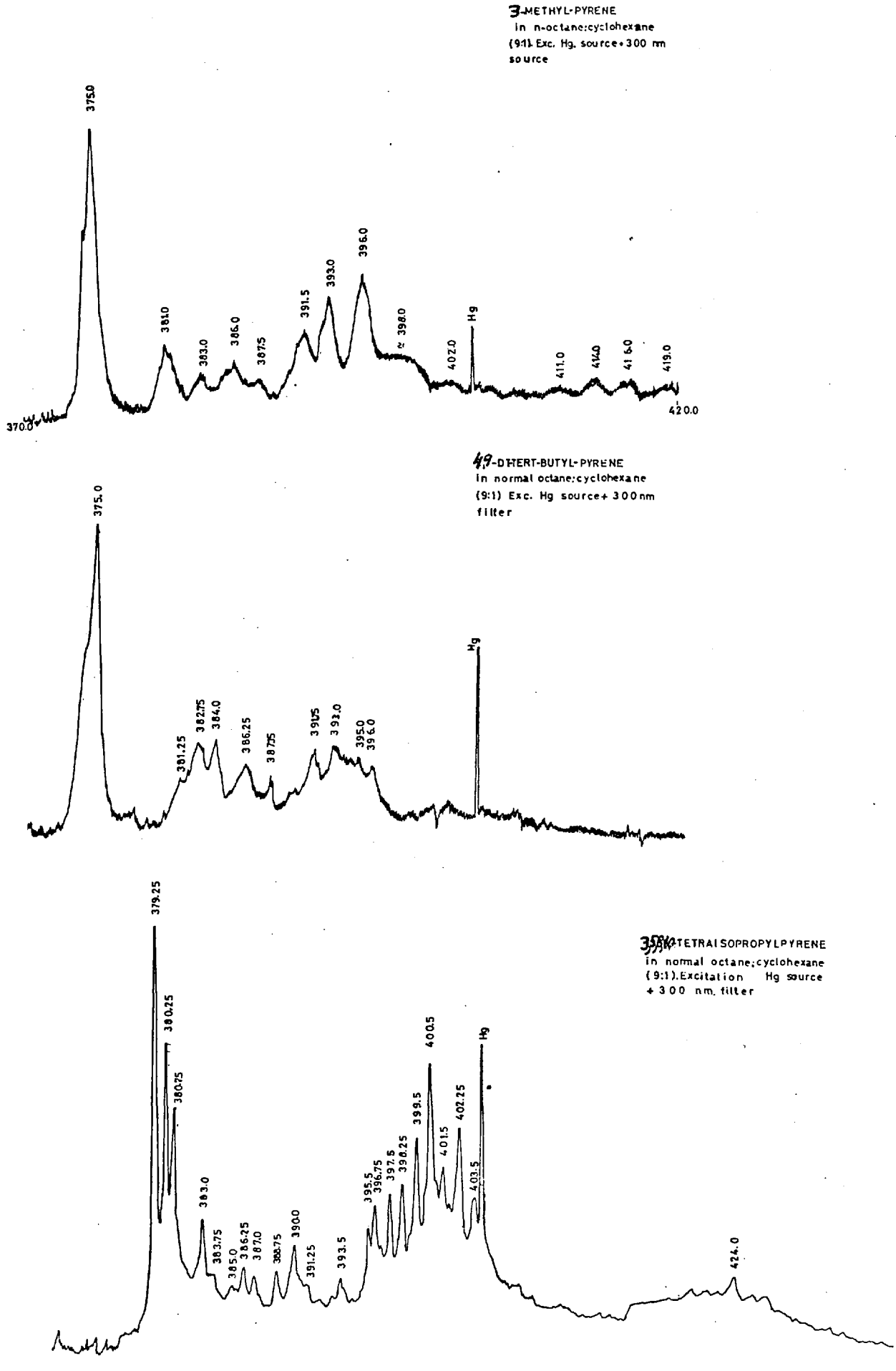

3-METHYL-PYRENE
in n-octane:cyclohexane
(9:1) Exc. Hg. source + 300 nm
source
375.0
381.0
383.0
386.0
387.5
391.5
393.0
396.0
≈ 398.0
402.0
Hg
411.0
414.0
416.0
419.0
370.0
420.0
4,9-DI-TERT-BUTYL-PYRENE
in normal octane:cyclohexane
(9:1) Exc. Hg source + 300 nm
filter
375.0
381.25
382.75
384.0
386.25
387.75
391.5
393.0
395.0
396.0
Hg
TETRAISOPROPYLPYRENE
in normal octane:cyclohexane
(9:1). Excitation Hg source
+ 300 nm. filter
379.25
380.25
380.75
383.0
383.75
385.0
386.25
387.0
388.75
390.0
391.25
393.5
395.5
396.75
397.5
398.25
399.5
400.5
401.5
402.25
403.5
Hg
424.0

Fig.IV-6

(Fig. IV-4), 3-methyl-pyrene, 4,9-di-t-butylpyrene (Fig. IV-6), phenanthrene and 9,10-dimethyl-anthracene, exhibit intense but broad band luminescence emission in n-octane-cyclohexane solvent. In these spectra typical half-widths greater than 1.0 nm were observed. Anthracene exhibited a very broad spectrum (Fig. IV-2b). Some of these compounds (phenarethrene, anthracene (Fig.IV 2A) and 9,10-dimethyl-antracene) were examined in n-hexane-cyclohexane solvent somewhat narrower half-band widths in their luminescence spectra (Fig. IV-4,5). 3,4,8,9-dibenzopyrene was examined also in n-decane-cyclohexane (90:10 V/V) and gave a more well-defined spectrum than that obtained in n-octane-cyclohexane. (Fig. IV.2d).

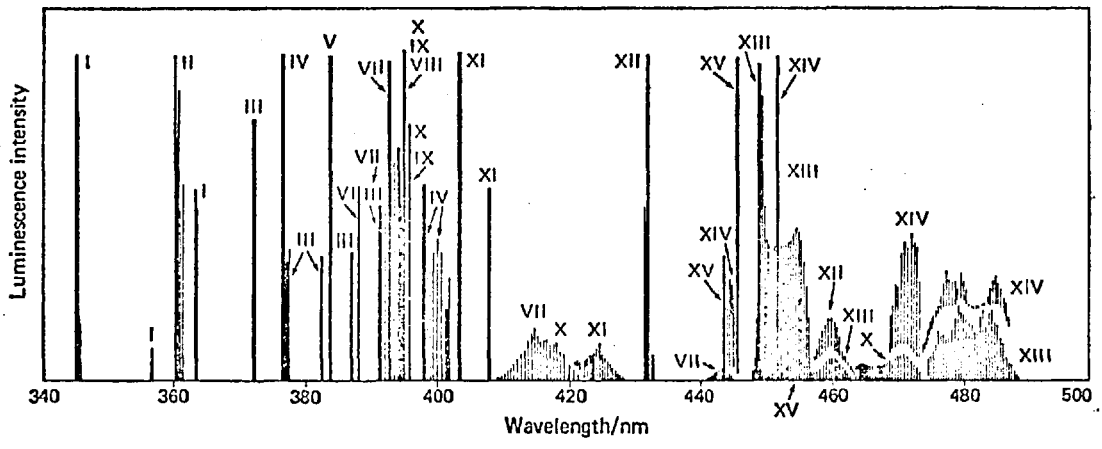


Fig. IV-7 Schematic presentation of the emission spectra at 77 K of some of the PAH compounds studied: (I) phenanthrene and (IV) anthracene in n-hexane - cyclohexane; (II) chrysene, (III) pyrene, (V) 1,2-benzanthracene, (VI) 1,2-benzopyrene, (VII) 3-methylcholanthrene, (VIII) 1,2,5,6-dibenzanthracene, (IX) 1,2,4,5-dibenzopyrene, (X) 1,2,3,4-dibenzopyrene, (XI) 3,4-benzopyrene, (XII) 3,4,9,10-dibenzopyrene, (XIII) 3,4,8,9-dibenzopyrene and (XIV) perylene in n-octane - cyclohexane; and (XV) coronene in n-hexane

A schematic presentation of the luminescence characteristic of fifteen of these compounds appears in Fig. IV-7.

Good agreement is observed in the wavelength assignments made for the principal quasi-linear luminescence emission maxima in this work with those recorded earlier for several of the compounds studied by other workers. A brief description of the luminescence of some of the compounds studied as follows.

Phenanthrene. (Fig. IV-5) presented a very weak and broad spectrum in n-octane-cyclohexane. Using a slit width of 0.1 mm, broad peaks appear at 364 and 346 nm. In n-hexane-cyclohexane, however, the Shpol'skii effect was observed. The emission maxima were located on a diffuse background. The Hg 253.7 nm was selected for excitation using a narrow band interference filter. Phosphorescence was also observed from phenanthrene in n-hexane-cyclohexane at 77 K between 455 and 510 nm. The luminescence of phenanthrene has been studied in n-pentane and n-hexane at 77 K by Ciais and Pesteil[159] and quasi-linear emission was observed in n-hexane at 77 K by Teplyakov[160]. In the following table a comparison is established between our values and the values of the most intense peak emission wavelength observed by Teplyakov. Good correlation was obtained.

Wavelength of Maximum Emission Intensity, nm

This work	459.25	468.0	477.0	496.25	-
From Ref. (160)	460.0	469.0	477.0	497.0	530

Triphenylene - only very weak fluorescence was observed for triphenylene in n-octane-cyclohexane. The phosphorescence emission is more intense, however, and several peaks have been assigned; the main peak is seen at 462.25nm (Fig. IV-5). This agrees well with the reported value of 461.8 nm for the triphenylene phosphorescence in n-hexane at 93 K observed by Bowen and Brocklehurst[18].

Perylene (Fig. IV.5) shows a well-defined narrow-line spectrum in n-octane-cyclohexane at 77 K. The wavelength of the principal emission maxima agrees well with those reported in n-hexane by Shpol'skii[24] and Bowen and Brocklehurst[18]:

This work (in n-octane:cyclohexane)	Ref(18) 93 K (in n-hexane)	Ref(24) 77 K (in n-hexane)
444.25	445.1	445.4
451.0	452.1	452.6
471.25	472.2	472.75
472.25	473.6	474.4
477.5	478.5	479.1

The small differences observed are probably due to the different n-paraffin employed by Shpol'skii[24] and Bowen and Brocklehurst[18] (n-hexane).

Pyrene - shows a well-defined quasi-linear emission spectrum in n-octane-cyclohexane (see Fig. III-2) and the wavelength assignments of the principal lines compare favourably with those reported by Klimova.[164]

1,2-benzopyrene - only weak emission was observed with 1,2-benzopyrene in contrast with the strong luminescence observed with 3,4-benzopyrene. In both cases the wavelengths in n-octane compare favourably with those previously reported. 1,2-benzopyrene showed the principal emission peak at 387.75 nm; some phosphorescence emission is also present at 537.25 nm. (Fig. III-2).

This work	387.75	396.25	407.25	409.25	-	410.5
Ref. (161)	387.62	395.95	407.44	408.85	410.14	410.63

The wavelength assignments are very similar to those reported by Shpol'skii, Klimova and Personov[161] in n-hexane at 77 K.

3,4-Benzopyrene - due to its biochemical importance, and being widely distributed in the environment, 3,4-benzopyrene has been widely studied and several papers have reported the quasi-linear luminescence characteristics of this compound[22,108]; our results agree quite well with those of previous data.

In order to demonstrate the fingerprinting ability of the technique, a synthetic mixture of eight PAH compounds was prepared and its low-

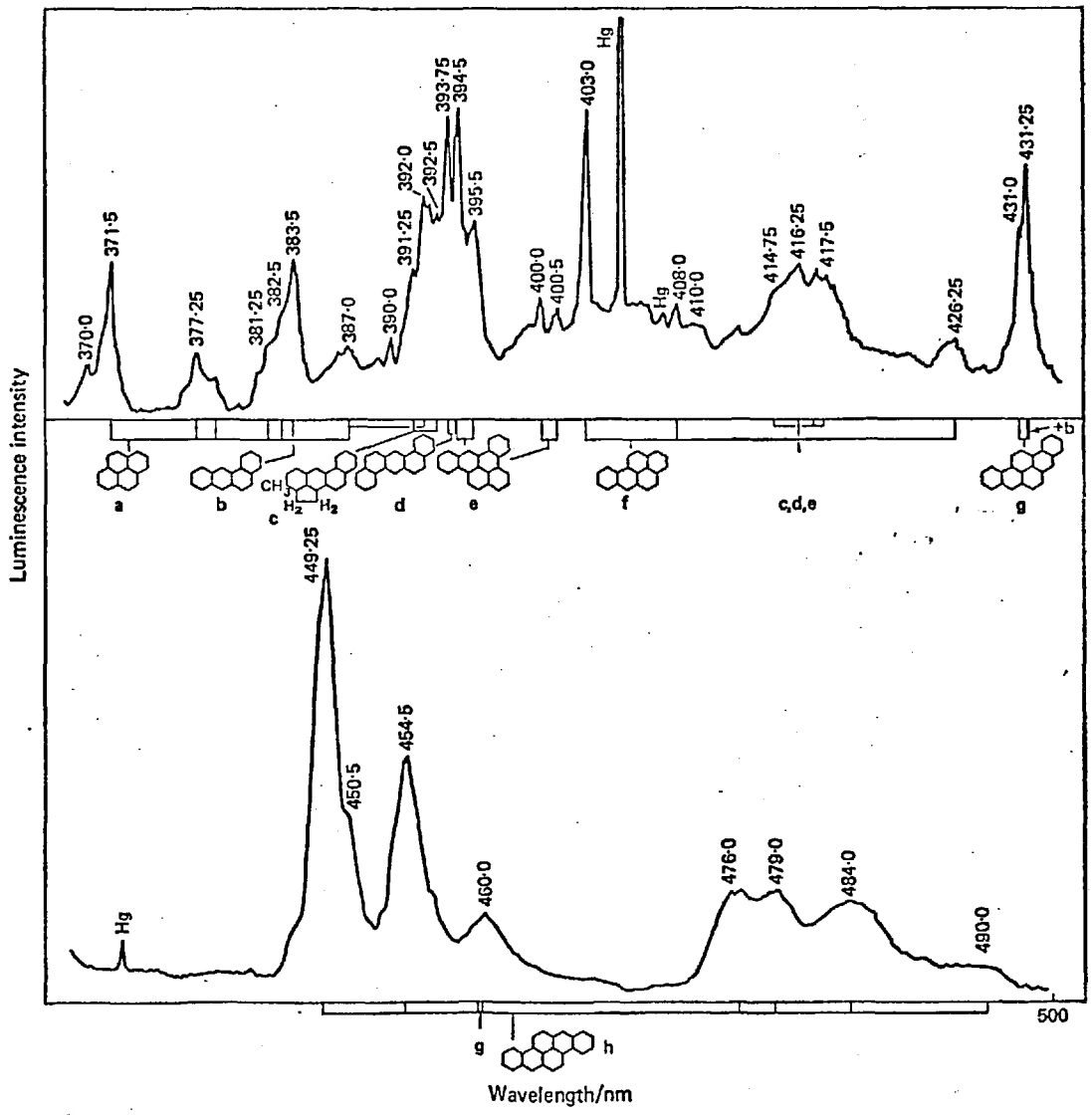


Fig. IV-8 Emission spectra of a synthetic mixture in n-octane - cyclohexane at 77 K: (*a*) pyrene; (*b*) 1,2-benzanthracene; (*c*) 3-methylcholanthrene; (*d*) 1,2,5,6-dibenzanthracene; (*e*) 1,2,4,5-dibenzopyrene; (*f*) 3,4-benzopyrene; (*g*) 3,4,9,10-dibenzopyrene; and (*h*) 3,4,8,9-dibenzopyrene

temperature luminescence emission spectrum was recorded at 77 K in n-octane-cyclohexane solvent; Fig. IV-8 shows the emission spectrum obtained. Each of the eight hydrocarbons present in the mixture is readily identified from the principal luminescence maxima observed. Even when some overlap occurs for certain principal peaks, there is sufficient information present in the minor features of the spectrum of each compound to permit its detection by using alternative peak wavelengths.

Practical Qualitative Applications

Qualitative examination of samples of soot and dust were undertaken. Although some experiments were also made or repeated with the monochromator described in chapter V they are included here for obvious reasons.

Soot - samples of soot were extracted with acetone and 0.1 ml of this extract was diluted to 1 ml with n-octane. The samples had a very strong blue fluorescence and a faint yellow phosphorescence. Scanning from 550 nm to 335 nm produced several peaks (Fig. IV-9). A group of emission lines at 371.85, 377.35, 382.25, 383.25 and 387.60 nm were identified from the aspect and similarity in wavelength as due to pyrene; the presence of 3,4-benzopyrene was easily recognised from the strong emission at 403.0 nm and also emission at 408.45, 423.75 and 426.75 nm. A peak at 419.40 suggested the presence of 1,12-benzoperylene. A group with doublets at 434.75, 435.30; 441.25, 441.95; and 462.05, 462.80 was also present. From the photographic spectrum published by Jäger[112] (Fig. III - 1) the presence of anthanthrene was suspected. This was tested by running a standard solution of anthanthrene in n-octane, when the presence of this compound was confirmed, this brought the problem of

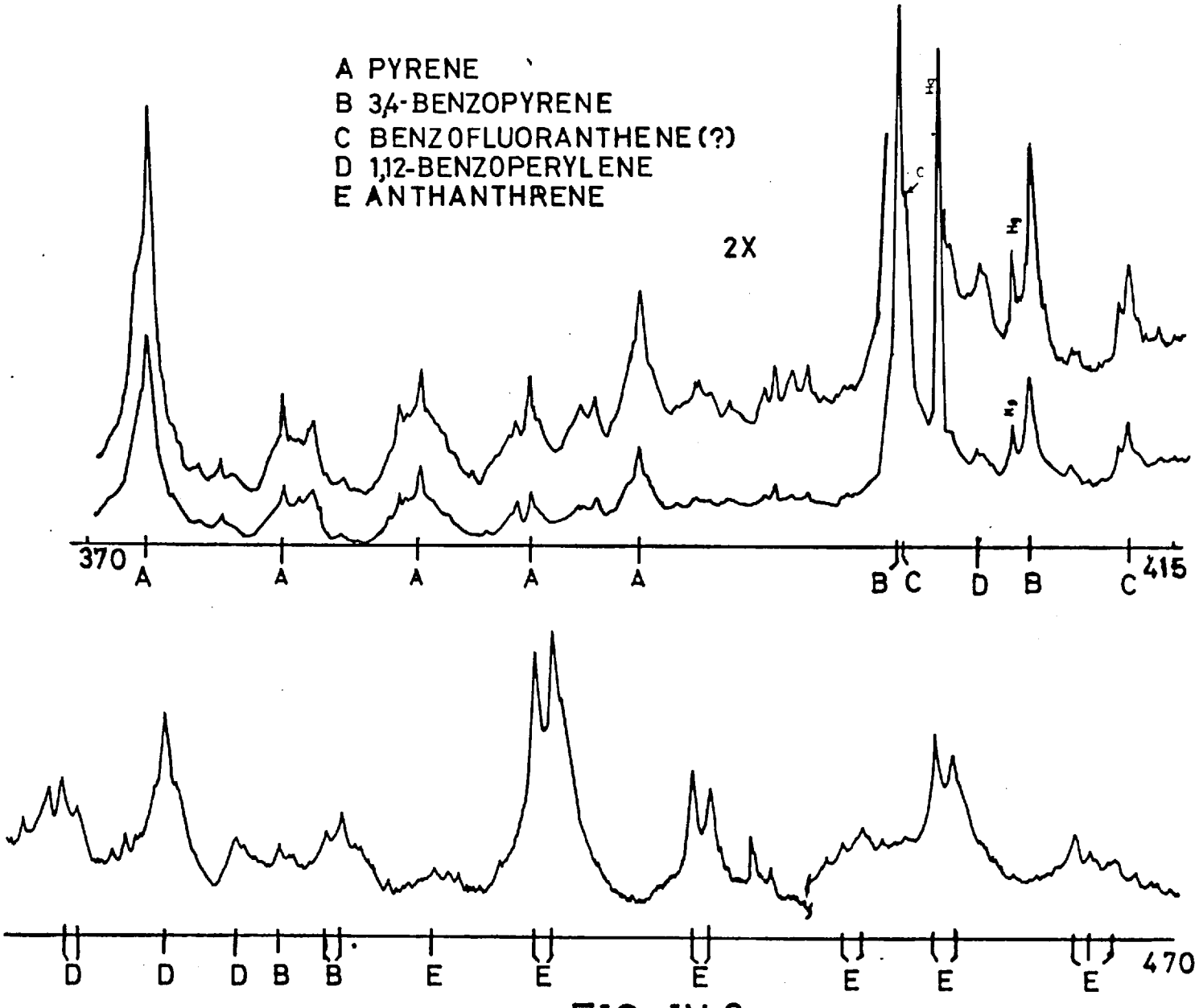

A PYRENE
B 3,4-BENZOPYRENE
C BENZOFLUORANTHENE (?)
D 1,12-BENZOPERYLENE
E ANTHANTHRENE
2X
370
A
A
A
A
A
B
C
D
B
C
415
D
D
D
B
B
E
E
E
E
E
E
470

FIG. IV-9

identification of a compound from a published photographic plate which is more difficult compared with a recording trace, as the photograph does not clearly show shoulders and small details.

A peak at ca. 403.40 suggested the presence of a 3,4-benzofluoranthene type of compound. Other weak peaks are resolved at 412.50 nm and ca. 428.0 nm which also suggest the presence of a benzofluoranthene type compound; 11,12-benzofluoranthene benzo(k)fluoranthene) is one of the probable compounds, however, as no standard was available no confirmation was possible.

Dust - dust was collected in the laboratory using a clean procelain evaporating basin (200 mm diameter) over a period of three months.

Sources of polycyclic aromatic hydrocarbons can be either external or internal. External sources are the pollution created by vehicular traffic around the laboratory and the pollution created by a distant power station. Internal sources can be rich-fuel Bunsen burners, tobacco smoke and in our case the products of combustion during analysis of organic sample using the carbon rod method of atomic fluorescence analysis. The black particulate material obtained had, however, the same appearance as the type of carbon dust present in the environment outside the laboratory.

10 mg of sample was the total amount of sample collected. This amount was extracted with 10 ml of cyclohexane over 3 hours with gentle heating in a flask in darkness to avoid photo-decomposition. The extract was concentrated to 1 ml by vacuum evaporation and 0.1 ml was diluted with either n-hexane or with n-octane for analysis.

3,4-Benzopyrene, 1,12-benzoperylene and again a probable a benzofluoranthene type compound were the only compounds detected. The analysis

done in n-hexane helped to identify 1,12-benzoperylene which was present in only a very small amount. 1,12-benzoperylene has a narrower emission in n-hexane than in n-octane and also a small shift in the emission provides another indication of the presence of this compound.

Other samples such as an acetone extract of sea mussel, an extract in cyclohexane of roasted chestnut and fractions of lubricant oil were also analysed. Due to the interference of other compounds present, or the prescence of the PAH compounds below the detection limit no positive identification was possible, although all of these sample exhibited very diffuse fluorescence or phosphorescence. In some of these cases preliminary sample clean-up by a chromatographic method of separation would probably have been beneficial to the analysis.

Conclusions

This study confirmed that a high selectivity and specificity is observed in the identification of PAH compounds utilising the Shpol'skii effect at 77 K. The quasi-linear emission spectra can be used for unambiguous 'finger-printing' of these compounds in solution at low concentration. The wavelength reproducibility of the emission maxima and the relative freedom from overlap of the very sharp lines observed compared with those obtained in solution at room temperature or at low temperature in glassy matrices show the principal advantage of the use of quasi-linear luminescence for analysis. The differences in spectral characteristics for a particular compound in different solvents is from our point of view an advantage in the characterization of an unknow compound.

Chapter V

Quantitative Application of Quasi-Linear Luminescence Spectrometry

Previous Work

The first semi-quantitative determination using a quasi-linear fluorescence emission spectrum was achieved in a study of the application of this method to the analysis of aromatic fractions of oils and bitumens by Il'ina and Personov[110] in 1960, when the concentration of perylene was estimated based on a comparison of the fluorescence spectrum of a n-hexane solution of a sample of oil at 77 K, with that of an artificial mixture made-up from a similar oil with a known concentation of perylene added.

Concomitantly Bogomolov, Pemova and Kolosova[113], in 1960, established a semi-quantitative method for the analysis of 3,4-benzopyrene in n-hexane at 77 K, based on the number of lines present in the spectrum; with 1×10^{2} µg ml^{-1} 21 lines were visible, while only 4 lines were observed at a concentration of 1×10^{-2} µg ml^{-1}.

Muel and Lacroix[108] devised the first quantitative method of analysis, using quasi-linear luminescence for 3,4-benzopyrene at 83 K, in n-octane employing standard addition. Using this method, Muel and Lacroix, obtained a precision of $\pm$ 10% and a detection limit of 1×10^{-4} µg ml^{-1}. The method of standard addition was used to avoid variations due to self-absorption, the effect of foreign ions and variation of the intensity of emission due to change in the freezing rate. Practical application was made by the use of this procedure to the determination of 3,4-benzopyrene in cigarette smoke, in spirits and in drinking water. 3,4-benzopyrene was found in significant amounts in cigarette smoke, however, in spirits, only trace amounts were found and in drinking water the amount

of 3,4-benzopyrene was undetectable.

The was soon followed by Dikun[114] who in 1961 devised a method for determining 3,4-benzopyrene at 77 K using 1,12-benzoperylene as internal standard using n-hexane as solvent. The proportionality between the lines was only observed at low concentrations (less than 4×10^{-6}M or $\simeq$ 1 μg ml^{-1}); the relative error for 15 analyses was $\pm$ 8 per cent. The method was compared with an absorption spectrophotometric method and a sensible improvement in sensitivity was observed with the new method - approximately 100 times higher.

The presence of 1,12-benzoperylene which is usually present in the same chromatographic fraction did not interfere as normally occurs in the absorption method.

Personov[115] independently devised a similar internal standard method in n-octane at 77 K. Anthracene, pyrene, perylene, 1,12-benzoperylene and coronene were tested as internal standard; perylene and coronene gave a fairly sharp fluorescence; as perylene, however, has a large number of intense absorption bands which distort the corresponding part of the fluorescence spectrum of 3,4-benzopyrene, coronene was used as internal standard.

The relative error was 7% in the concentration range 5×10^{-2} to 5×10^{-3} μg ml^{-1}. Using a fraction which had 2.3×10^{-2} μg ml^{-1} from a sample of carbon black the analytical precision was about 15%.

Personov also observed that the ratio $I_{3,4\text{-benzopyrene}}/I_{\text{coronene}}$ was extremely sensitive to changes in the freezing rate; also if the sample had fluorescence quenchers which modified the intensity of 3,4-benzopyrene and the intensity of coronene in a different way this was observed to lead to changes in the relative intensity of their lines.

Using this method (internal standard) Prokhorova and Znamensky[133] determined 3,4-benzopyrene in paraffin of Soviet production; in a certain brand a concentration of 2.6×10^{-3} μg ml^{-1} of 3,4-benzopyrene was found.

Eichoff and Köhler[121] investigated the luminescence characteristics of anthracene, 1,2,5,6-di-benzanthracene, 3-methylcholanthrene and 3,4-benzopyrene at 79 K in n-heptane; with the latter the curve of intensity versus concentration was obtained. The relative error was 6.4% at a concentration of 1 μg ml^{-1} and ca 15% at a concentration of 1×10^{-3} μg ml^{-1}; the detection limit was 6.7×10^{-4} μg ml^{-1}. An analysis of particulates from air was made using this absolute intensity method and a concentration ranging from 0.05 to 0.1 g/1000 m^3 of air was found.

Personov and Teplistskaya[118], using the absolute intensity method and the method of additions determined 3,4-benzopyrene, perylene and 1,12-benzoperylene in organic material from minerals and rocks. The detection limit obtained for the pure compounds in n-hexane was 1×10^{-5} μg ml^{-1} for 3,4-benzopyrene and 3×10^{-5} μg ml^{-1} for both perylene and 1,12-benzoperylene. Using a real sample a comparison was made between the direct method and the method of standard additions and very similar results were obtained for both perylene and 1,12-benzoperylene. The relative standard deviation for both methods was also very similar, in the range 10 to 20%.

Personov and Teplitskaya[118], however, raise the point that if the sample is known not to quench the fluorescence and that strict requirements are not imposed on the magnitude of the error the analysis can be, in such case, carried out using the method of comparison with

a standard solution rather than by the means of the more accurate method of standard addition.

Varshavskii, Shabad, Khesina, Khitrovo, Chalabov and Pakhol'nik[116] used the method of Muel and Lacroix of standard additions for the analysis of 3,4-benzopyrene (at 77 K in n-octane) in diesel exhaust gases. The detection limit was 1×10^{-4} μg ml^{-1} and the error did not exceed 10%.

Jäger and Lugrová[111] applied the method of standard additions for the determination of 3,4-benzopyrene in samples of solid particles taken from the exhaust of cars (using petrol) under different operating conditions. In a second paper Jäger[127] also analysed anthanthrene and 1,12-benzoperylene in exhaust gases. The amount of carcinogenic material produced was a function of the load, age and speed of revolution of the motor. Jäger and Kassowitzova[162-3] determined the concentration of 3,4-benzopyrene in drinking water and the photochemical modifications of 3,4-benzopyrene, in water, in the presence of light or other chemical compounds. These analyses by Jäger and co-workers were undertaken using n-heptane as solvent at 77 K. Jäger and Lugrová also compared the method of quasi-linear luminescence analysis using standard additions with the classical absorption method: in all cases the fluorescence technique showed results with suppressions in a range from 3 to 8%.

Synthetic mixtures also showed that the amount of 3,4-benzopyrene found after analysis was always less than the amount added (-7 to - 10%). The authors explain this effect as caused by interference from the other compounds present in the mixture. Also it was found that the high concentration limit for 3,4-benzopyrene analysis was 0.15 μg ml^{-1} in the final solution being optimum at a (typical) value of 0.05 μg ml^{-1} (ca 2×10^{-7} M).

Florovskaya, Teplitskaya and Personov[119] continued their previous work[118] on the analysis of polynuclear aromatic hydrocarbons (such as perylene, 1,12-benzoperylene and 3,4-benzopyrene in organic material from rocks and mineral samples of different origin, using an n-hexane matrix.

Danil'tseva and Khesina[120] established a method for the analysis of 9,10-dimethyl-1,2-benzanthracene in n-octane at 77 K. The method used was a combination of both standard addition and internal standard (combined method); 3,4,5,6,7-tri-benzopyrene was chosen as for the internal standard as this compound has a sharp quasi-linear fluorescence spectrum in n-octane. The relative error was not more than $\pm$ 10% and the detection limit was 1 $\mu g\ ml^{-1}$.

Dikun, Kranitskaya, Gorelova and Kalinina[123] compared the combined method described above with the internal standard method and the method of additions for the analysis of 3,4-benzopyrene in n-octane at 77 K. As standard for the internal standard and for the combined method 1,12-benzoperylene was used.

Large spread in the results was obtained when the method of additions was used ($\pm$ 29%) and similar results (8-10%) were obtained when either the internal standard or the combined method was employed.

Dikun and co-workers[123], however, raise the point that when real samples are being analysed it is possible that they include other substances that could quench the fluorescence signal of the compound being studied. According to Personov and Teplitskaya[118] such compounds - although they present no real problem in either the combined method or in the method of additions - can interfere in the internal standard method. Assays of 3,4-benzopyrene, by Dikun and co-workers,[123] in

different samples using internal standard or combined method show that the results exhibit discrepancies but it was not possible to come to a definite conclusion that the impurities present in the samples were responsible for the difference; these workers concluded that the danger from quenching agents in the internal standard method appears to have been greatly overestimated by Personov and Teplitskaya[118]

Fedoseeva and Khesina[124] investigated the conditions for the quantitative analysis of the eight polycyclic aromatic hydrocarbons; 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, 1,2,5,6-dibenz-anthracene, 1,2-benzopyrene, 3,4-benzopyrene, pyrene, perylene and 1,12-benzoperylene using the combined method (addition and internal standard method). 1,12-benzoperylene was used as internal standard when the methodsof analysis for 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, 3,4-benzopyrene, pyrene and perylene were established; 3,4-benzopyrene was used as internal standard for the establishment of the method of analysis for 1,2,5,6-dibenzanthracene, 1,2-benzopyrene and 1,12-benzoperylene. A relative error of 3% was observed, when ten measurements were made, but in a single analysis the relative error reached 10%. The sensitivity of the method was a function of the individual characteristics of the compound, (such as quantum efficiency), the selected analytical line and the sensitivity of the spectrometer and the photomultiplier response.

The sensitivity varied from 1×10^{-2} µg ml^{-1} (in the case of 9,10-dimethyl-1,2-benzanthracene and 1,2-benzopyrene) to 1×10^{-3} µg ml^{-1} (in the case of pyrene, 1,2-benzanthracene and 1,2,5,6-dibenzanthracene). In the case of 1,12-benzoperylene, perylene and 3,4-benzopyrene the

sensitivity was better than 1×10^{-4} μg ml^{-1}. An investigation was also made by these workers using all possible paired combinations of the substances in synthetic mixtures, using a molar ratio of 1:1 (interference: analyte) and a concentration of 1×10^{-1} μg ml^{-1} (except perylene which was 1×10^{-2} μg ml^{-1}) and in these cases the maximum error did not exceed 10%.

Kolyadich, Khesina, Shefer and Yamskova[125] applied the combined method to the study of the formation of 3,4-benzopyrene in industrial oils during their use. The limit of detection was 5×10^{-3} μg g^{-1} and the error was less than 10%. n - Octane was used as solvent and liquid nitrogen was used as coolant. The concentration was a function of the age of the oil. After seventeen hours the 3,4-benzopyrene content increased from an undetectable amount to 7,9 μg g^{-1}.

Stepanova, Il'ina and Shaposhnikov[129] determined the content of some polynuclear aromatic hydrocarbons, using the Shpol'skii effect, in exhaust gases, dusts, soots and effluents from different chemicals plants, as kerosene pyrolysis, eletrolytic aluminium, phenolformaldehyde, bitumen and cardboard production units. 3,4-benzopyrene, 1,12-benzoperylene, 1,2-benzanthracene, 1,2,5,6-dibenzanthracene and 1,2,3,4-dibenzanthracene were analysed in the samples using the combined method[120]. Using a synthetic mixture a relative error of 15-17% was obtained with the compounds analysed. Due to lack of standard, 1,2,3,4-dibenzanthracene was quantitatively estimated from the absolute intensity reading at 387.9 nm. In this case the error in the synthetic mixture was +42 per cent.

Khesina and Petrova[130] using also the combined method and n-octane as solvent, determined 3,4-benzopyrene, pyrene, 1,2-benzopyrene,

1,12-benzoperylene, 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, perylene and coronene in waste water; 0.5, 14.0, 1.0, 0.15 and 0.3g l^{-1} were respectively found for the former compounds, but no concentration was reported for 1,2,5,6-dibenzanthracene, perylene and coronene.

Biological applications using quasi-linear luminescence have been described by Fedoseeva and Khesina[124] as oxidation of carcinogenic hydrocarbons by bacteria and yeast, by action of living cells and also the effect of soil microflora in the destruction of 3,4-benzopyrene.

As we can see the quasi-linear luminescence method is already a routine method of analysis in some laboratories in particular in the Soviet Union. Recently in the Proceedings of the Plenary session of the Committee on the Carcinogenic Substances (of the board of the Ministry of Public Health of USSR)[134], Khesina, Dikun, Shabad and Serkovskaya, together or independently, presented several reports in which methods of analysis based in the Shpol'skii effect were described or recommended for the analysis of carcinogenic polycyclic aromatic compounds - principally 3,4-benzopyrene - in the environment, in fish and meat products, in petroleum derivatives, in phenolformaldehyde plastic, waste gases, etc.

Methods of Separation used in Conjunction with the Quasi-linear Luminescence Method of Analysis

Chromatography has been used by most of the workers during the analysis of real samples. Serkovskaya[134] employed a primary separation (clean-up) by column chromatography in silica-gel, followed

by thin layer chromatography in aluminium oxide for the separation of fractions from samples of petroleum and petroleum derivatives.

Personov[115] utilized a chromatographic fraction in the evaluation of the method of internal standards; however there are no other details in the published paper. Eichhoff and Köhler[121] used paper chromatography to isolate fractions, firstly from a synthetic mixture (anthracene, 3,4-benzopyrene, 1,2,5,6-dibenzanthracene and 3-methylcholanthrene; 100:1:1000:100) and later from a sample of airborne particulate material.

Varshavskii, Shabad, Khesina, Khitrovo, Chalabov and Pakhol'nik[116] observed that in their case the need of chromatography was eliminated by the use of the method of additions; combustion tars and soots were the typical samples. Jäger, Lugrová[111] and Kassowitzova[162, 163] used thin layer chromatography in aluminium oxide. Less error was observed when chromatography was used (-20%) in comparison with the direct analysis (-40%) using a synthetic sample.

No other specific method of separation was used by Florovskaya, Teplitskaya and Personov[117] after the extraction of the PAH from rocks and minerals samples.

Danilt'seva and Khesina[120] recommended chromatography when the impurity interferes with the substance being analysed or with the compound used as standard. Lavalette[109] and co-workers admitted that in a mixture of seven compounds, five components could be detected, if selective excitation is used.

Dikun, Kranitskaya, Gorelova and Kalinina[123] applied quasi-linear luminescence spectrometry when complex products and chromatographic procedures were employed but there are no other details

concerning the exact method which was used.

Fedoseeva and Khesina[124] observed that if impurities quench the luminescence of the investigated compound or if they luminesce or create a continuous background, it is impossible to carry out the analysis without prior chromatographic separation. According to these workers the separation is, however, not complete and polycyclic aromatic hydrocarbons with similar molecular weight will usually be present in the final fractions.

Kolyadich, Khesina, Shefer and Yamskova[125] used aluminium oxide column chromatography together with aluminium oxide thin layer chromatography as methods of separation before analysis.

Stepanova, Il'ina and Shaposhnikov[129] adopted thin layer chromatography (aluminium oxide) as a clean-up procedure.

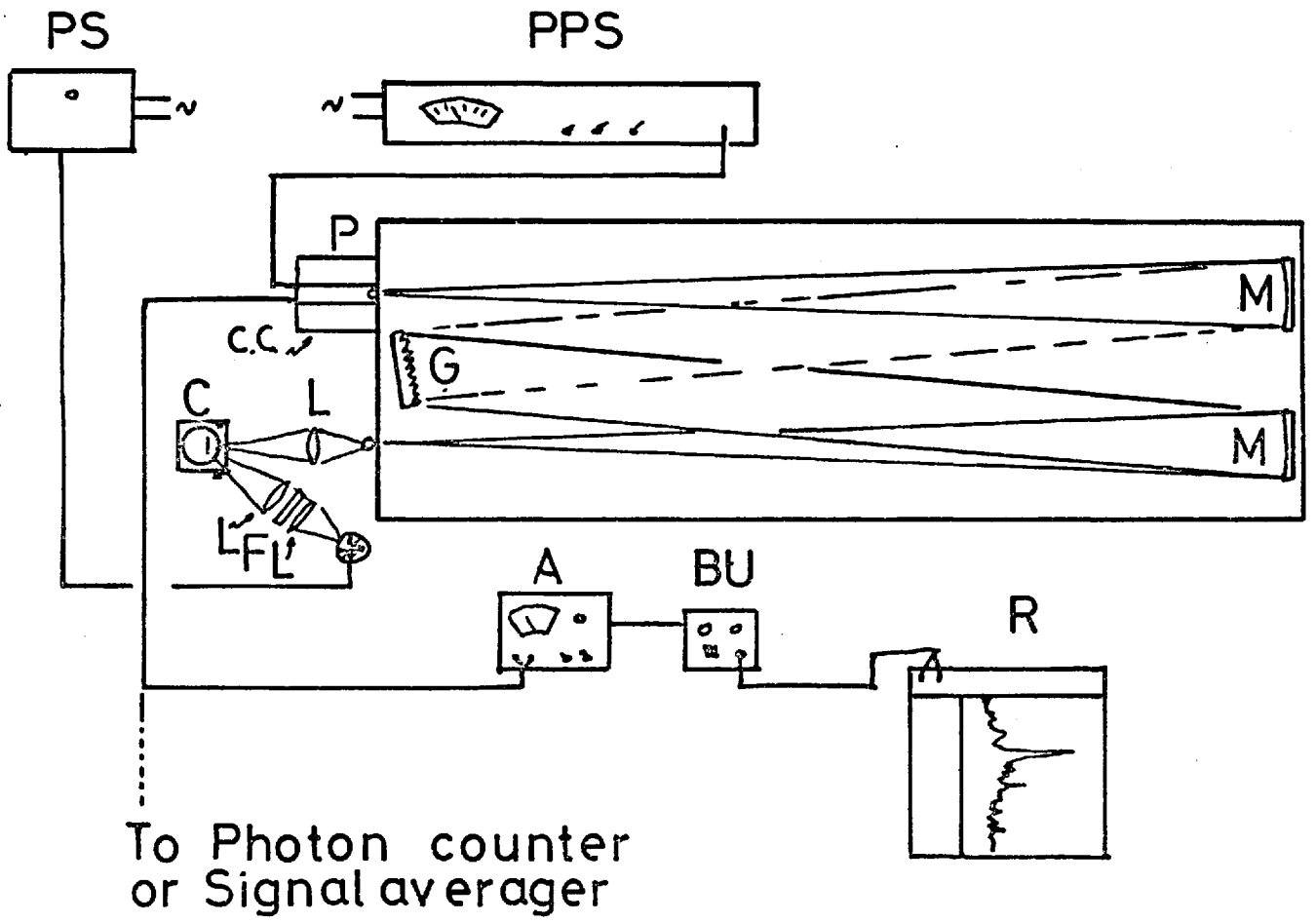
Khesina and Petrova[130] partially isolated the polycyclic aromatic hydrocarbons from real samples using an alumina column and gradient elution with n-hexane and benzene.

Experimental

Instrumentation

For the quantitative studies the instrumental arrangement described in Chapter IV was initially employed; an improved instrumental arrangement (Fig. V-1) described below was developed during the course of this work.

A monochromator of greater resolution than the earlier system was used. This monochromator (Rank-Hilger Monospek 1000, with an



PS.power supply
PPS.photomultiplier power supply
P-photomultiplier
M.mirrors
G-grating
C-cell
L-lenses
F-filter
A-amplifier
BU-backing-off unit
R-recorder
c.c.-cooling chamber

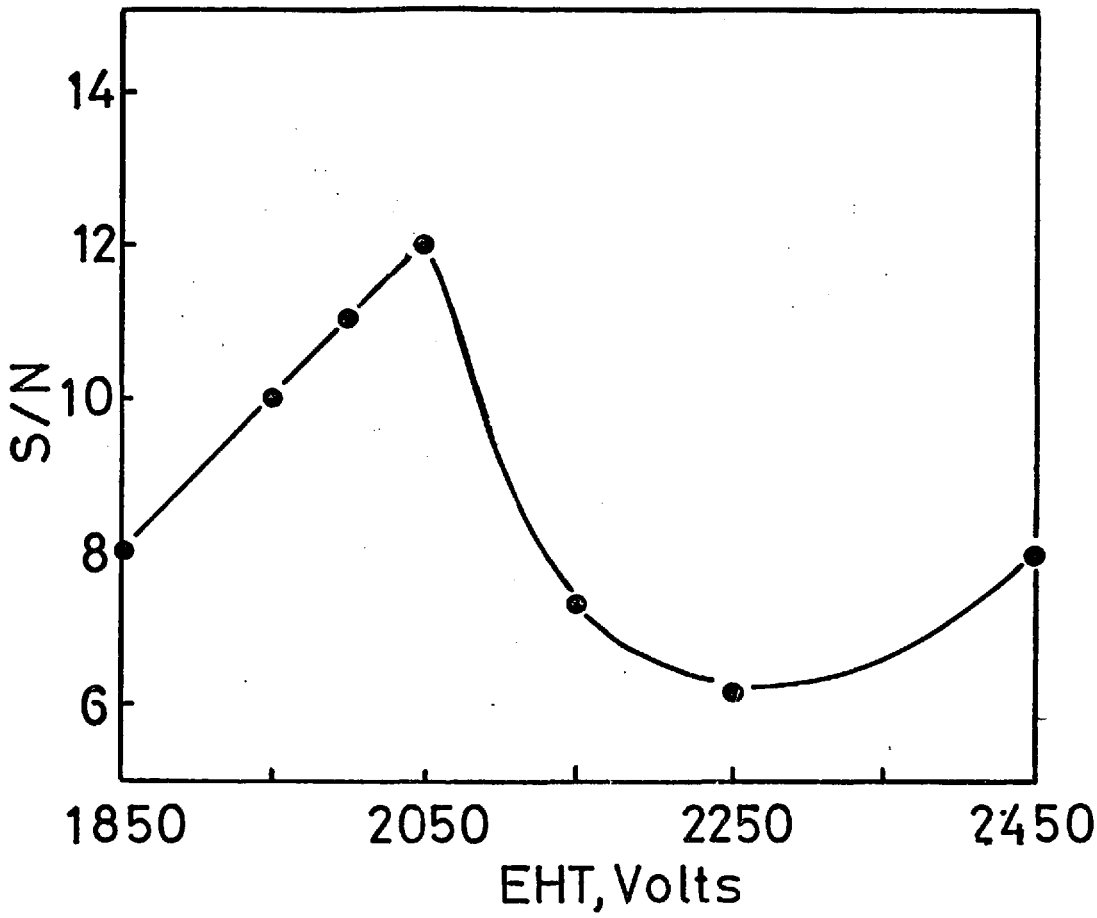
FIG. V-1

aperture of f/8) is a plane grating, symmetrical, Czerny-Turner assembly in which the radiation transmitted though the entrance slit is collimated and directed towards the diffraction grating by a collimating mirror. The diffraction grating (Jobin-Yvon, 1200 lines mm^{-1} blazed at 300 nm) disperses the incident radiation and part of the dispersed radiation falls on to a focussing mirror which directs and focusses a spectrum onto the exit slit. The diffraction grating was rotated either manually or by a motor drive; a choice of 10 drive velocities (from 0.05 nm min^{-1} to 50 nm min^{-1}) was available. A velocity of 5 nm min^{-1} was used in most of the experiments. The linear reciprocal dispersion of this monochromator with the grating employed is 0.82 nm mm^{-1} at the exit slit. Both entrance and exit slits were independently adjustable with a micrometer drum: each division of the micrometer wheel represented 0.005 mm. The length of the slit was also adjusted by the use of a adjustable diaphragm and the Hartmann number was indicated on a scale. Both slits were provided with shutters, placed behind the slit.

A 50 mm EMI 6256S photomultiplier, which has 10 mm of effective cathode area, was used as detector. This photomultiplier is characterized by an 'S' type response and has a Spectrosil (fused silica) window (which extends the useful range down to about 165 nm) and has a very high gain (ca.10^8) and low dark current[157]. The photomultiplier was attached to the exit slit of the monochromator. A cooling chamber was constructed around the photomultiplier housing to be used with dry ice in the photon counting experiments, described in a later chapter.

The optimum operating voltage (2050 V) was found from the curve of signal-to-noise ratio versus applied voltage (Fig. V-2). As before a Brandeburg EHT supply was used.

A high pressure mercury vapour discharge (125 watts, Philips MBW/U) was used as excitation source. The Wood's glass envelope was



Signal to noise ratio vs. voltage, using EMI 6256S, $1X10^{-9}$ M 3,4-Benzopyrene, and a copper cryostat.

Fig. V-2

removed from the lamp and the source was the driven from the mains through a laboratory-made ballast unit. The angle between the excitation and emission beams was ca 45° and frontal illumination was consequently used in this arrangement. The lens system was the same as that used in the previous arrangement. The light from the source was focussed onto the

monochromator entrance slit.

The signal was either recorded directly at a potentiometric chart recorder (Servoscribe, Model RE 511.20) or was first amplified using a microammeter (RCA, Model WV-84C) and then recorded. As a very large background was present in most experiments a laboratory-made backing-off unit was used for compensation.

Quantitative Analysis Experiment

A study was made with the quasi-linear emission of four dibenzopyrene compounds available - 1,2,3,4-dibenzopyrene, 1,2,4,5-dibenzopyrene 3,4,8,9-dibenzopyrene and 3,4,9,10-dibenzopyrene. These four dibenzopyrenes are compounds of biological interest due to their strong carcinogenic activity[165] and some of these compounds have also been detected in the environment of some cities of the United States[166], Russia[122] and Hungary[167].

The variation in intensity of the luminescence emission at 77 K with concentration was examined using these four dibenzopyrenes in n-octane: cyclohexane (9:1 v/v) and the intensity of the quasi-linear emission for 3,4,9,10-dibenzopyrene, 1,2,4,5-dibenzopyrene and 1,2,3,4-dibenzopyrene was measured at 431.50, 395.50 and 395.25 nm respectively (Fig. V - 3a, b and c). The variation of intensity of luminescece emission for 1,2,3,4-dibenzopyrene was also measured at its broad emission peak at 470.25 nm. Measurement at this wavelenth, rather than at 395.25 mn, would be advantageous in the determination of this compound in the presence of 1,2,4,5-dibenzopyrene as it avoids interference from the emission of the latter compound at 395.5nm. The luminescence growth curves obtained

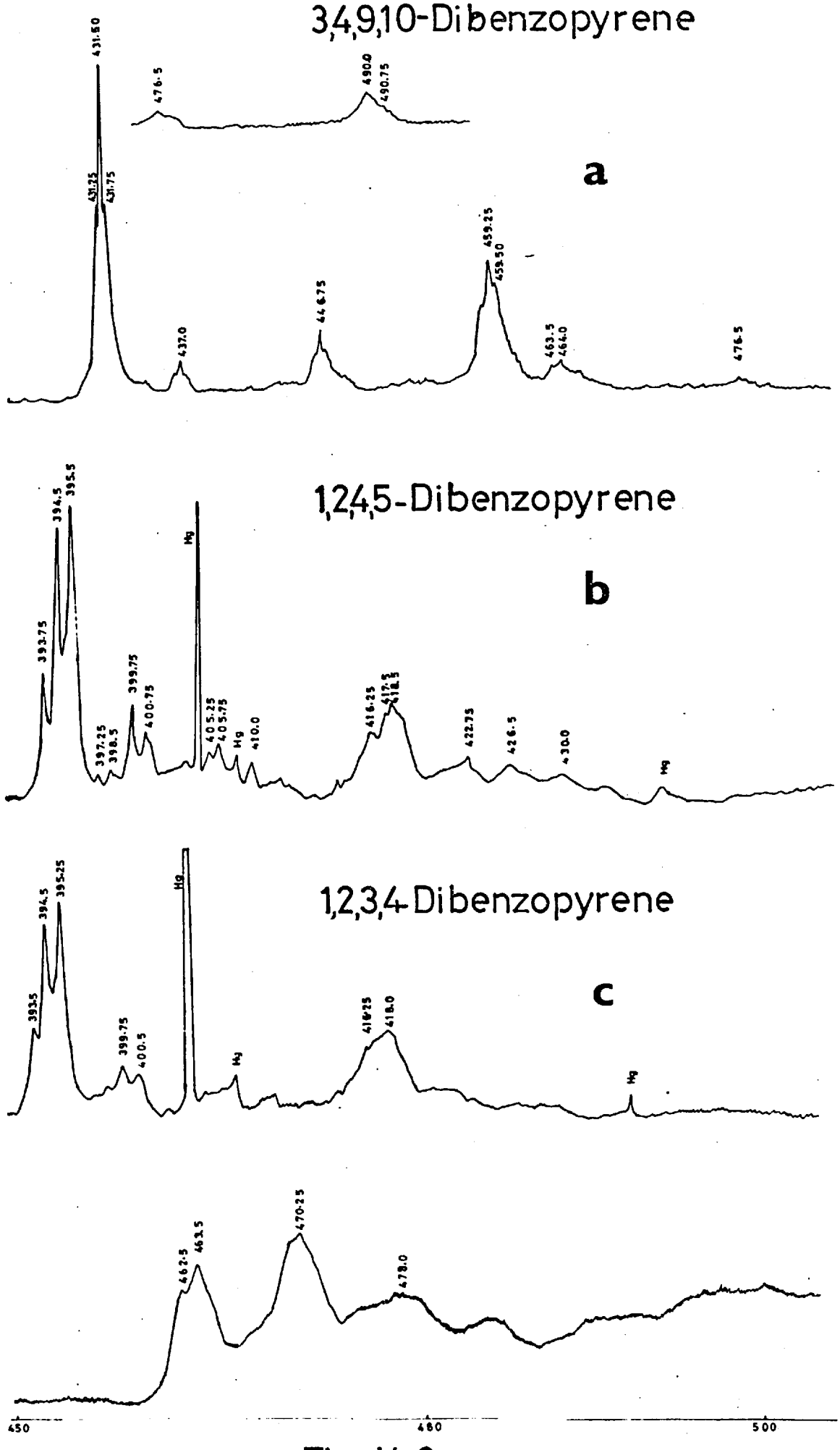
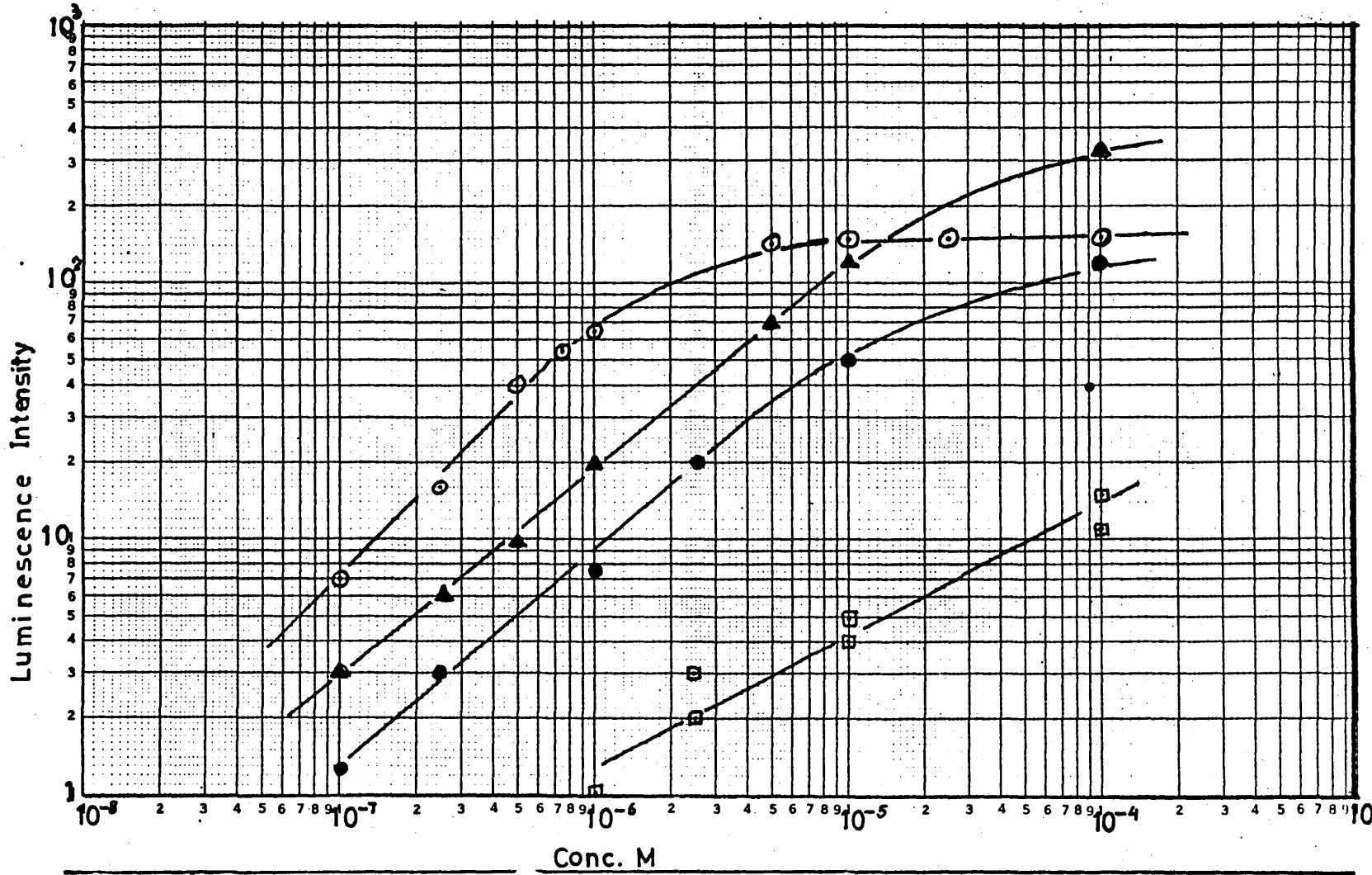

3,4,9,10-Dibenzopyrene
a
1,2,4,5-Dibenzopyrene
b
1,2,3,4-Dibenzopyrene
c
Hg
450
460
500

Fig. V-3



⊙3,4,8,9-DIBENZOPYRENE; △3,4,9,10-DIBENZOPYRENE; ●1,2,4,5-DIBENZOPYRENE.
⊡1,2,3,4-DIBENZOPYRENE

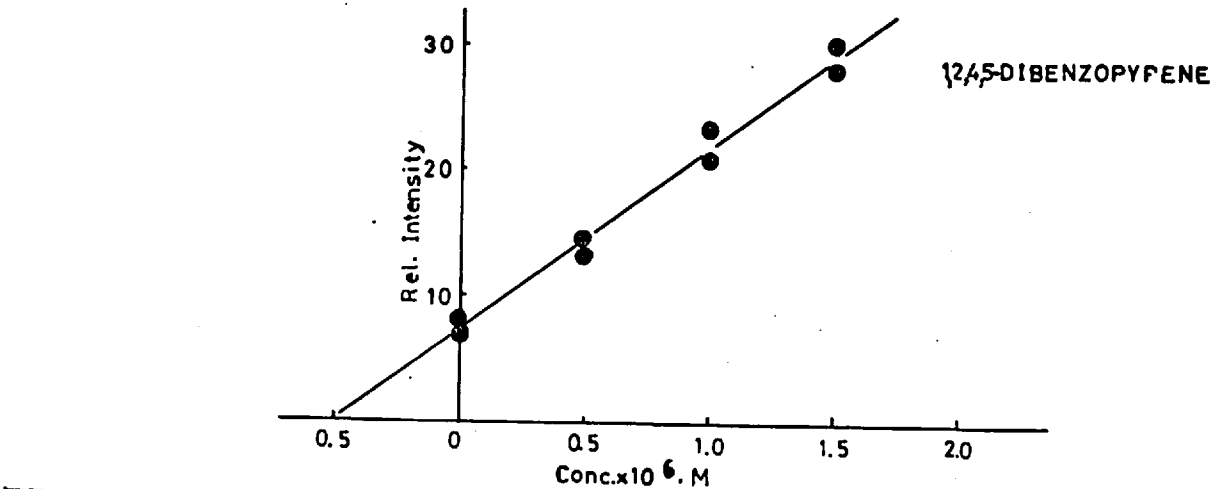
Fig. V-4

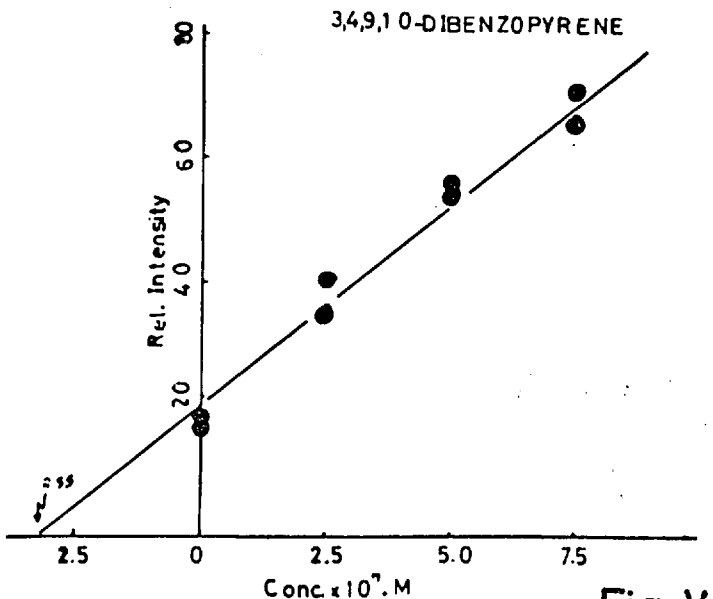
are plotted logarithmically in Fig. V-4.

For the compounds examined, the quasi-linear emission intensity is linear over a concentration range of two orders of magnitude. Fig. V-4 also shows that similar intensity, slope and range of linearity are obtained at both 395.25 and 470.25 nm for the luminescence growth curve of 1,2,3,4-dibenzopyrene. Although 3,4,8,9-dibenzopyrene does not show quasi-linear luminescence emission in n-octane-cyclohexane solvent at 77 K, it can be seen from fig. V-4 that a similar intensity and range of linearity is observed for the luminescence of this compound when measured at the wavelength of its more intense emission at 449.25 nm(Fig.IV-2c).

Although it appears that direct measurement of the intensity of quasi-linear luminescence emission is capable of permitting the direct quantitative determination of compounds such as the dibenzopyrenes, as already quoted in the introduction of this chapter, there are possibilities of quenching and variation in the experimental conditions such as rate of freezing, or the reproducibility with which the sample cell can be placed in the optical path. These effects can be minimised by the use of an internal standard in quantitative work, assuming however, that the differences in freezing rate affects in an equal manner both the compound being analysed and the compound being used as internal standard.

The study of the four dibenzopyrene compoundswas made using the combined standard addition-internal standard procedure, for the quantitative determination of the dibenzopyrene compounds in n-octane-cyclohexane (9:1 v/v). The internal standard employed was selected so that the wavelength of its quasi-linear emission did not overlap that of the compound to be determined. It is, of course, not possible to avoid such overlapping in the corresponding excitation spectra; it is necessary


1,2,4,5-DIBENZOPYRENE
Rel. Intensity
30
20
10
0.5
0
0.5
1.0
1.5
2.0
Conc.x10 6. M


3,4,9,10-DIBENZOPYRENE
Rel. Intensity
60
40
20
2.5
0
2.5
5.0
7.5
Conc.x10⁷. M

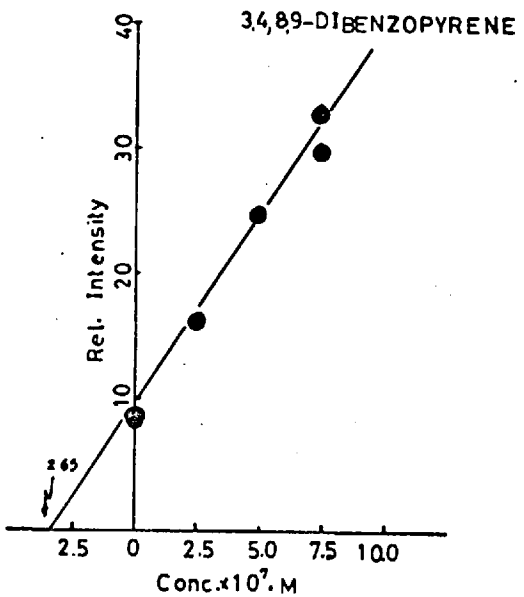

3,4,8,9-DIBENZOPYRENE
Rel. Intensity
40
30
20
10
2.5
0
2.5
5.0
7.5
10.0
Conc.x10⁷. M

Fig. V-5

for radiation transmitted by the single filter used to excite luminescence from both analyte compound and internal standard, so that some overlap in excitation spectra must occur if the internal standard technique is to be used in this manner.

1,2,4,5-Dibenzopyrene, 3,4,9,10 and 3,4,8,9-dibenzopyrene were determined by measurement of their quasi-linear luminescence in n-octane-cyclohexane at 77 K at the wavelengths employed in order to construct their luminescence growth curves shown in Fig. V-4. The quasi-linearemission of 3,4-benzopyrene at 403.0 nm was used as internal standard for the determination of 1,2,4,5-dibenzopyrene and 3,4,9,10-dibenzopyrene and a standard additions procedure was employed in order to produce the calibration graphs shown in Fig. V-5(a) and (b). For the determination of 3,4,8,9-dibenzopyrene in n-octane-cyclohexane the quasi-linear emission of 1,2-benzopyrene at 387.75 nm was used for internal standardisation; the calibration graph obtained is shown in Fig. V-5c. In each instance the ratio of the observed intensities for the luminescence of the analyte and internal standard are plotted against concentration.

Detection Limits

The detection limits given for most of the compounds studied can be defined as that concentration of hydrocarbon in the solvent employed which gives a signal-to-noise ratio of 2 at the wavelength of the principal quasi-linear emission. The wavelength in nm used, and the values in $\mu g\ ml^{-1}$ obtained for these compounds are shown

below. Some of the detection limits were also determined using the definitive instrumentation described in Instrumentation in this chapter and are presented in Table V-1.

Table V-1

Compound	Wavelength nm	Det. limit[1] $\mu g \cdot ml^{-1}$	Det. limit[2] $\mu g \cdot ml^{-1}$
Phenanthrene	345.5	1×10^{-1}	-
Triphenylene	462.25	1×10^{-1}	-
Chrysene	360.5	5×10^{-2}	-
Perylene	451.0	5×10^{-2}	1×10^{-2}
Pyrene	371.75	5×10^{-2}	5×10^{-3}
3,4-Benzopyrene	403.0	5×10^{-3}	1.25×10^{-4}
1,2-Benzopyrene	387.75	7×10^{-2}	1×10^{-3}
1,2,3,4-Dibenzopyrene	395.25	5×10^{-2}	3×10^{-4}
1,2,4,5-Dibenzopyrene	395.5	1×10^{-1}	1×10^{-3}
3,4,8,9-Dibenzopyrene	449.25	1×10^{-2}	1×10^{-4}
3,4,9,10-Dibenzopyrene	431.5	4×10^{-2}	3×10^{-4}
Anthracene	377.0	5×10^{-2}	-
1,2-Benzanthracene	383.75	1×10^{-1}	2×10^{-3}
9,10-Dimentyl-1,2-Benzanthracene	397.75	2×10^{-1}	3×10^{-3}

1. Preliminary instrumental arrangement (Chapter IV)theoretical band width 0.16 nm.
2. Final instrumental arrangement (Chapter V) theoretical band width 0.08nm.

3-Methyl-cholanthrene	392.5	2×10^{-1}	5×10^{-3}
1,2,5,6-Dibenzanthracene	394.25	1×10^{-1}	5×10^{-3}
9,10-Dimethylanthracene	405.75	5×10^{-2}	-
3-Methyl-pyrene	375.0	2×10^{-1}	-
4,9-Di-t-butyl-pyrene	375.0	2×10^{-1}	-
3,5,8,10-Tetraisopropyl-pyrene	379.25	1×10^{-1}	-
2,3-O-Phenylenepyrene (Indeno[1,2,3-cd]pyrene)	462.5	3×10^{-1}	-
1,2-Benzonaphtho [2',3':4,5]pyrene	444.75	3×10^{-1}	-
Coronene	445.0	1×10^{-1}	1×10^{-3}
3,4-Benzofluoranthene	403.25	-	1.5×10^{-3}

The enhancement of sensitivity when the final instrumental assembly was used in relation to the former is due to several factors. Firstly a stronger excitation source was used in the later assembly (125 watts) in comparison with the previous assembly (50 watts) and as there is a well known linear dependence between the intensity of the fluorescence signal and the intensity of the exciting source, which follows the equation V-A[158]

$$F = I_0(2.3 \epsilon c \ell)\emptyset \qquad \text{V-A}$$

which is valid for very dilute solutions in which only a small fraction of the incident light is absorbed and where F - total fluorescence intensity (in quanta per unit time); I_0 - the intensity of the exciting light (in quanta per unit time); ϵ - molar absorptivity of the fluorescing solute; c - molar concentration of the solute, ℓ - path-length of solution (in cm) and ϕ - the quantum efficiency of fluorescence.

The second important factor was the use of a pre-amplifier (the RCA microammeter) which has an amplification factor of 60.

Other factors that contributed are the difference in the angle of illumination employed; as the solution is polycrystalline it is not transparent to the excitation beam. The smaller aperture of the monochromator, the gain and the response type of the photomultiplier used in the latter arrangement also influenced the detection limits obtained. A smaller theoretical band width is used with the final instrumental arrangement; if equal band width is used a much better sensitivity would be observed with the final instrumental arrangement.

Other alternative sources of excitation were also tested and evaluated. A xenon arc lamp, a helium-cadmium laser and a microwave-excited electrodeless discharge tube were investigated; the results are described below.

a) Xenon Arc Excitation

In some experiments a high intensity d.c. xenon arc lamp (Osram XBO W/1 with 150 watts), which produces a continuum from 200 to 600 nm was employed. The lamp was driven by a Perkin-Elmer 150 watt power supply, which provides an output of 20 volts d.c. The exciting radiation was isolated using the same interference filters as before. 3,4-benzo-

pyrene and coronene were used as test compounds and an excitation filter which isolates the radiation around the 300 nm region was used. Alternatively a low resolution spectrometer (Hilger 242) was used to isolate the exciting radiation. Using the 300 nm filter the detection limit was half of that observed using the 125 watts mercury vapour discharge lamp; with the monochromator the situation was even worse: the limit of detection was about 1/100. We also observed that in the region far from the region isolated by the filter or the monochromator, less scattered light was present. Using a 300 nm interference filter and at wavelengths less than 405 nm, a continuum, which increases as the wavelength decreases, was observed (Fig. V-6). The image of the source

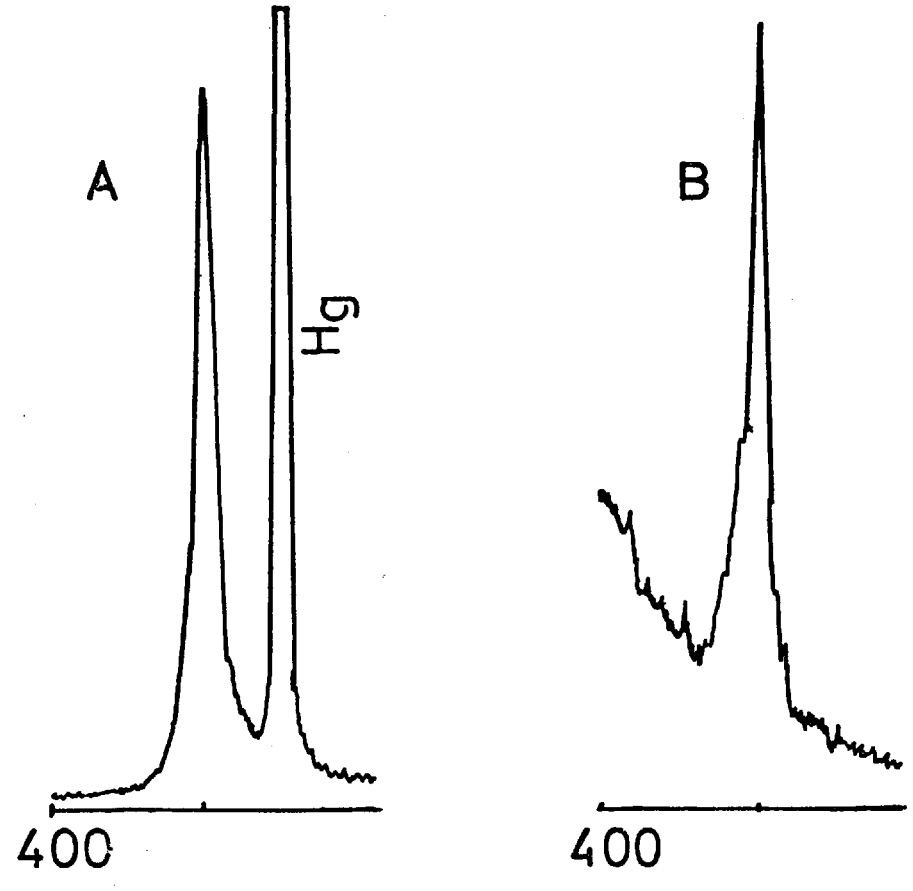


Fig. V-6. A. Using a mercury vapour discharge lamp (Philips MBW/U 125 W); B. Using xenon arc lamp (Osram XBO 150 W). Quasi-linear emission of 3,4-benzopyrene in n-octane at 77 K.

projected in the sample is point-like and less sample is therefore needed (about half of the amount needed when the mercury lamp is used) which decreases the absolute detection limit.

In the region where the background is more intense - when approaches the region transmitted through the filter - the signal-to-background ratio is smaller if compared with the mercury lamp; at 400.0 nm the background observed is ca. 5 times more intense using the xenon lamp.

With a solution of 3,4-benzopyrene (in n-octane) with a concentration of 1×10^{-7}M the signal to background at 403.0 nm was 19:1 with the mercury lamp and only 2:1 with the xenon lamp. Where the filter transmits more intensely, the background slope distorts the spectrum. The lack of scattered lines however, creates a condition to the observation of quasi-linear emission in position where the mercury scattered lines overlap the emission. An example of such a problem was observed in the case of the weak quasi-linear emission of diphenylene oxide in tetrahydrofuran (see Chapter VIII) where emission at 434.10 was obscured by the scatter of the strong mercury line, creating doubt about the presence of the quasi-linear emission; the use of xenon lamp excitation solved this problem and the quasi-linear emission at 434.10 nm was confirmed.

For compounds which are best excited by the 253.7 nm mercury line the xenon lamp did not produce good results; perylene, for instance, showed an emission using xenon excitation which was 10 times weaker. Nevertheless we believe that an instrumental arrangement must have both types of excitation sources (mercury and xenon) available, as in

some particular cases the best performance will probably be found with one of these sources.

b) Laser Excitation

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As it is our belief that a tunable dye-laser will ultimately be the best source for the excitation of compounds which emit quasi-linear luminescence, an evaluation was made using a helium-cadmium laser (Electro-Photonics/Liconix, Model 401/301) which was available for a short period. This laser has the possibility to emit in two distinct wavelengths (325.0 nm and 441.6 nm) by changing appropriate mirrors in the system.

Table V-2 and Table V-3 show the results obtained with this laser in comparison with results obtained with the Philips MBW/U mercury vapour discharge lamp (125 watts).

Table V-2 shows that, when the 325 nm mode was used, although no real advantage was observed in the signal intensity when laser excitation was in use in this case, the signal to noise ratio is higher in most of the cases - such as with coronene and 1,2-benzanthracene - and to a lesser extent with 1,2,5,6-dibenzanthracene. Both low noise and low background were observed with the laser excitation using the 325.0 nm mode. Scattered weak peaks were observed in the wavelength range between 375-500 nm. If an interference filter is used to isolate the 325.0 nm line, a strong reduction in the intensity of the quasi-linear emission is observed, the signals being reduced to 15-20% of their original intensity.

Table V-3 shows that, when the 441.6 nm laser was used, only in the case of perylene was an enhancement of both signal and signal to noise ratio observed; this is due to the fact that the 441.6 nm line

Table V-2

Compound	Conc. $\mu g\ ml^{-1}$	Lum. Signal (325.0 nm Laser) mV	Lum. Signal (mercury)[1] mV
Coronene[2]	7.5	60	77
(at 445.05 nm)		S/N 1000	S/N 513
3,4-Benzopyrene[3]	0.3	55(8.5)[4]	128
(at 403.0 nm)		S/N 916(141)	S/N 853
1,2-Benzanthracene[3]	2.0	156(34)[4]	98
(at 384.4 nm)		S/N2600(566)	S/N 653
1,2,5,6-Dibenzanthracene[3]	2.0	58	88
		S/N 966	S/N 586
Typical Background (at 400.0 nm)		0.14	0.50
Typical Noise (at 400.0 nm)		0.06	0.15

1. Mercury vapour-discharge lamp with a 300 nm interference filter.
2. In n-hexane solvent.
3. In n-octane solvent.
4. Values in parenthesis refer to experiments done with an interference filter(maximum at 325.0 nm). S/N. Signal to noise ratio.

Table V-3

Compound	Conc. $\mu g\ ml^{-1}$	Lum. Signal (441.6 nm Laser) mV	Lum. Signal (mercury) mV
Perylene[1] (at 443.95 nm)	0.25	1400 S/N 700	15[2] S/N 150
3,4,8,9-Dibenzopyrene[3] (at 449.15 nm)	0.30	800 S/N 400	284[4] S/N 2800
Ovalene[1] (at 501.6 nm)	12	21 S/N 10.5	3.8[5] (6.4)[6] S/N 38(64)
Typical Background (at 501.0 nm)		5	0.5
Typical Noise (at 501.0 nm)		2	0.1

1. Using n-hexane as solvent.
2. Using either the 250 nm or 325 nm interference filter.
3. Using n-octane as solvent.
4. Using either the 300 nm or the 325 nm filter.
5. Using the 300 nm filter.
6. Using the 325 nm filter.

is exciting exactly (i.e. overlap) the longest-wavelength 0-0 band of the absorption spectrum of perylene[29].

In the other cases although ca. 3 times enhancement in signal is observed, the signal-to-noise ratio did not increase in the same proportion, as more noise was observed when the laser was used with the 441.6 nm mode.

Nevertheless, these preliminary experiments suggest that if a tunable dye laser is used and the compound can be excited properly substantial enhancement in the signal-to-noise ratio will be observed and in consequence an increase in the sensitivity of the method and probably the selectivity, as it is well known that a quasi-linear absorption spectrum is usually present together with the quasi-linear emission[69,97].

c) Electrodeless Discharge Lamp Excitation

Using 3,4-benzopyrene solution (0.2 μg ml^{-1}) in n-octane, two electrodeless discharge lamps were tested as excitation sources. Bismuth and aluminium EDL were used and a 300 nm interference filter was employed to isolate the emission at 306.8 nm (bismuth) or the emisssion at 308.2 and 309.3 nm (aluminium).

The fluorescence intensity was, however, 10 times weaker when the EDL were in use in comparison with the 125 watts mercury vapour-discharge lamp.

Quantitative Practical Application

Using the direct method and the combined method (standard addition-internal standard) analyses of 3,4-benzopyrene were made in used lubricant oil. Used lubricant oil is a sanitation problem, principally when it is inadvertently disposed of in the environment. In some countries the oil is recovered and probably will be more and more as the price of raw material increases.

The samples were analysed without any previous clean-up or separation procedures. All samples were obtained from the sumps of vehicles of ages between 5-8 years and which had had regular oil changes. The samples were homogenized by agitation before weighing.

10 mg of each oil sample was weighed. This was dissolved in 5 ml of n-octane. From this master solution other solutions were prepared for the direct method and the combined method. Coronene was used as the internal standard (using the emission at 444.45 nm). Table V-4 shows the results obtained using the direct method and the combined method. The base line method was employed to obtain the net values. In all cases lower results were obtained when the direct method was employed, showing the quenching effect caused by the

TABLE V-4

Sample	Direct Method ($\mu g\ ml^{-1}$)	Combined Method ($\mu g\ ml^{-1}$)
KR81	ca. 9.5	21 ± 2
MO12	ca. 2.5	6.5 ± 0.5
NY83	ca. 9.5	25 ± 5

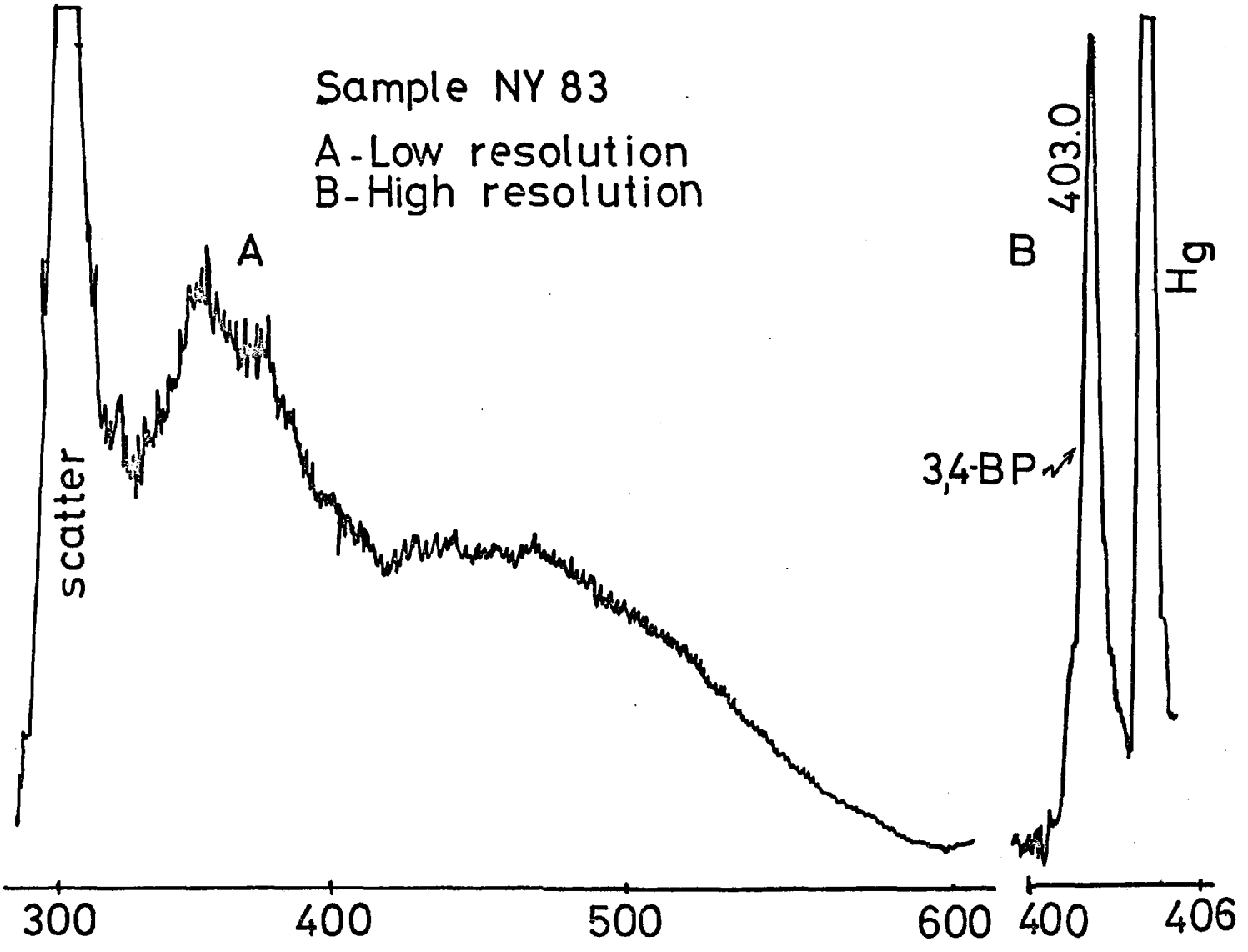

Sample NY 83
A-Low resolution
B-High resolution
A
B
403.0
Hg
3,4-BP
scatter
300
400
500
600
400
406

FIG. V-7

presence of other compounds. In all samples a strong blue phosphorescence was observed, which was, however, diffuse in aspect.

In two samples (MO12 and NY83) a very weak shoulder at 403.30 suggested the presence of a benzofluoranthene type compound, probably 11,12-benzofluoranthene, which was not available as a standard.

As a comparison one of the samples was analysed using the low resolution double monochromator spectrofluorimeter (Aminco). However, no 3,4-benzopyrene signal was observed, although attempts were made with different excitation wavelengths and only a broad emission was observed.

This is due to the low resolution of the instrument where the weak 3,4-benzopyrene peak is not resolved from the noise observed in the spectrum (Fig. V-7).

Chapter VI

The Quenching of Quasi-Linear Luminescence

Wehry[168] has described in a concise manner the possible ways in which the fluorescence of an organic molecule can be affected by the solute-solute interactions. In this description, Wehry divided considerations of fluorescence quenching into a) - processes in which electronic energy transfer is possible (such as the inner-filter effect, collisional non-radiative transfer and long-range non-radiative transfer) and b) - processes not involving electronic energy transfer (such as charge-transfer quenching). Oxygen quenching and the external heavy-atom effect was described by Wehry separately[168].

Quenching of quasi-linear luminescence has been observed by some workers, and as already described in Chapter V, the standard addition or combined method, together with prior separations of interferents, have been recommended to solve such problems. Some fundamental studies concerning this phenomenon have also been undertaken by Russian researchers.

a. Quenching by similar compounds

Dobrokhotova, Kul'chitskii and Naboikin[169] studied the possibility of energy transfer using naphthalene as donor and di-(9-anthryl)-1,2-ethylene (DAE) as the acceptor molecule. Such system is usually employed as liquid scintilator when toluene is used as solvent, but in this study n-hexane was used as solvent. The DAE was maintained at constant concentration and the concentration of naphthalene was progressively increased (0.1; 1.0 and 10 moles/mole of DAE). By selective excitation of the naphthalene, the luminescence intensity of DAE increases with the naphthalene/DAE ratio, showing the energy

transfer from the naphthalene to the DAE molecule. The DAE line spectrum disappears when energy transfer is present. These workers believe that naphthalene molecules are frozen together with DAE molecules so that any short range order is probably similar to that in liquid solution.

Klimova and Nersesova[54,170], in order to obtain supplementary information about the isolation of a molecule (anthracene, as donor) added small amounts of a second molecule as acceptor, always selecting the added compound so that its excited electronic level lies below that of the first excited state of the compound under investigation (the donor). Naphthacene was used by Klimova and Nersesova as acceptor[170]. At low concentrations (1×10^{-6} M) the components of the mixture do not interact with one another and give characteristic quasi-linear spectra. In this case, the spectrum of the mixture is a superposition of the quasi-linear spectra of anthracene and naphthacene. As the anthracene concentration is increased (1×10^{-5} M - 1×10^{-2} M) energy transfer is observed from the anthracene molecules, which form aggregates, to the naphthacene molecules located in their vicinity. According to Klimova and Nersesova[170], as a consequence of the energy transfer, the diffuse fluorescence spectrum of anthracene (which is attributed to the emission of aggregates) decreased in intensity and at the same time a long wave-length emission spectrum appears which was found to correspond to that of the sensitized fluorescence of the acceptor.

Sometimes, the intensity of the fluorescence band spectra, however, decreases when an acceptor is introduced, and at the same time diffuse bands appear in the fluorescence spectrum of the mixed crystal and also weak bands appear in the absorption spectrum.

According to Shpol'skii, Klimova, Nersesova and Glyadkovskii[55] the phenomena are probably caused by partial aggregation of the donor molecules due to the introduction of an acceptor. Klimova, Nersesova, Naumova, Oglobina and Glyadkovskii[54] observed that the fluorescence and absorption band spectra also belong to single molecules which, however, unlike the molecules which produce quasi-linear spectra find themselves in conditions that do not preclude the possibility of contact with acceptor molecules whose presence in the solution can significantly affect their state. Using a laser excitation together with sensitized fluorescence Klimova et al[54] demonstrated that energy transfer does not take place between molecules responsible for the quasi-linear spectra.

b. Oxygen quenching

Teplitskaya and Personov[171] studied the influence of oxygen on the luminescence of 1,12-benzoperylene, 3,4-benzopyrene, perylene and coronene in n-hexane at 293 and 77 K. At 293 K a very strong oxygen quenching was observed; at 77 K, however, the quenching is absent. This indicates that the oxygen present in the solutions during the analytical determinations, does not affect the results. The absence of quenching confirms that the molecules are inside the micro-crystal, isolated from the oxygen[171]. Additionally the solubility of oxygen in n-hexane is very low at 77 K.

Grebenshchikov and Personov[172] studied coronene, triphenylene, biphenyl and diphenylene oxide.

With coronene in n-octane and in n-dodecane above 100 K oxygen

quenching was observed. At 77 K no quenching was observed[173]. The phosphorescence of these compounds was quenched by oxygen only if a temperature above 90 K was used; at this temperature gas diffusion is presumably possible[173].

c. The external heavy-atom effect and quasi-linear luminescence

Bolotnikova and Sichkar[174,175] studied the effect of external heavy atoms on the quasi-linear spectra using ethyl bromide as additive. Singlet-singlet absorption and fluorescence, triplet-triplet absorption and phosphorescence(and the phosphorescence lifetime) were examined. Benzene and naphthalene in cyclohexane, pyrene in n-decane and in n-hexane, and phenanthrene and diphenyl in n-hexane were the compounds studied. When ethyl bromide (at a concentration of 1.5 M) was introduced into the solutions of naphthalene, phenanthrene and pyrene (concentration 1 x 10^{-2} M) broadening of the triplet-triplet absorption bands was produced and an increase in the phosphorescence signal intensity was also observed. In the case of naphthalene the phosphorescence signal intensity was found to increase by a factor of 15; the spectrum was, however, less distinct and displayed towards shorter wavelenth ca. 100 cm^{-1}.

With pyrene (in n-hexane and in n-decane) at a concentration of 1 x 10^{-2} M, the phosphorescence spectrum remained unchanged in the form of a quasi-line spectrum; the only modification was the lifetime which decreased from 0.6 to 0.25 sec. The quasi-linear phosphorescence spectrum of phenanthrene (at a concentration of 1 x 10^{-2} M in n-hexane solvent) also remained unchanged; the lifetime, however, decreased from 3.3 to 0.7 sec. Almost similar behaviour was observed with 1M benzene in cyclo-

hexane; the lifetime decreased from 1.2 to 0.6 sec.

With benzene, toluene and naphthalene new singlet-singlet absorption bands did appear, with the addition of ethyl bromide. This led Bolotnikova and Sichkar to conclude that a charge-transfer complex had been formed[174]. Also the same authors[175] observed that the optimum concentration value for ethyl bromide was 10%, when the largest increase in the phosphorescence intensity was observed with naphthalene, pyrene, phenanthrene and diphenyl.

d. Effect of ethyl alcohol

During the studies undertaken by Bolotnikava and Sichkar[176] they observed that ethyl alcohol affected the absorption spectrum of naphthalene in cyclohexane (using a concentration of 1×10^{-2} M) in a similar manner as ethyl bromide. Shifts were similar (ca. 160 cm^{-1}) and it was suggested that the same type of complexes were formed. The appearance of the band of the complex when alcohol was added, was, according to these workers, unexpected since the amount of alcohol present was very small (10% in volume)[176].

Experimental

a. Quenching experiments with dibenzopyrene compounds

During the preliminary quantitative experiments done with the four dibenzopyrenes available to us (Chapter V), the effect of the addition of a second compound was studied. Table VI-1, shows the effect of the presence of equimolar or greater concentration of some other PAH compounds on the quasi-linear luminescence intensities observed for the

TABLE VI-1 Effect of other PAH compounds on Measured Luminescence Intensities at 77° K for Dibenzopyrenes Compounds.

INTERFERENCE COMPOUND	1,2,3,4-DBP	1,2,4,5-DBP	3,4,8,9-DBP	3,4,9,10-DBP	3,4-BP	1,2-BP	1,2,5,6-DBA	PER.[e]
1,2,3,4-Dibenzopyrene	- - -	+226[a]/nil[b]	-35[a]/nil[b]	-35[a]/nil[b]	-54.0[a;b]	- -	+22.2[a]/[g]-29.6[b]	—
1,2,4,5-Dibenzopyrene	-34.5	- - -	-35.0	-57.2	-60.5	- -	-22.9/f	--
3,4,8,9-Dibenzopyrene	-37.8	-33.4	---	-14.9	-58.0	-45.5[c]	--	-22.8/-35.8[d]
3,4,9,10-Dibenzopyrene	-17.8	-31.5	-45.8	---	-20.6 -56.2[d]	- -	-22.8 -32.8[d]	-31.5 -82.5[d]

(a) at 395.5 nm (b) at 470.5 nm (c) 4-fold excess of 1,2-BP (d) 10-fold excess of PER. (e) Perylene is not excited with the filter employed. (f) at 10-fold excess there is direct interference. (g) at 10-fold excess there is direct interference in the emission at 395.5 nm and suppression (-60%) at the 470.0 nm emission.

3,4-Benzopyrene, 1,2-benzopyrene, 1,2,5,6-dibenzanthracene and perylene appear in the table respectively as 3,4-BP, 1,2-BP, 1,25,6-DBA and PER.

four dibenzopyrenes excited at 300 nm, with mercury vapour discharge lamp (50 watts) and using an interference filter. As solvent was used n-octane: cyclohexane (90:10 v/v). In these experiments the instrumental arrangement described in Chapter IV was used.

The percentage suppression or enhancement of the luminescence signal in the presence of these compounds is listed. The concentration of the dibenzopyrene were in all experiments 5×10^{-6} M. In almost all instances suppression of quasi-linear emission of the dibenzopyrene occurs. The enhancement of the 1,2,3,4-dibenzopyrene emission at 395.5 nm in the presence of 1,2,4,5-dibenzopyrene is caused by the direct overlap of their quasi-linear emission at this wavelength; no effect is observed when the luminescence is measured at 470.5 nm.

b. Quenching experiments with 3,4-benzopyrene

Further experiments were done, using the instrument described in Chapter V and utilising 3,4-benzopyrene as analyte, in n-octane solvent, at 77 K. Phenanthrene, pyrene, 3,4-benzofluoranthene, anthanthrene, 1,12-benzoperylene and perylene were used as interferents and the effects observed are described as follows.

Phenanthrene - no effect was observed for phenanthrene - 3,4-benzopyrene concentration ratios of 1:1, 10:1 and 100:1, using a range of concentration from 5×10^{-8} M to 1×10^{-4} M of 3,4-benzopyrene. At concentrations of phenanthrene larger than 5×10^{-5} M, the emission of phenanthrene was also observed.

Pyrene - with pyrene: 3,4-benzopyrene concentration ratio of 1:1 no interference was observed, At 10:1 a weak background starts to appear at 403.0 nm and a percentage suppression on the signal of 9-15% was observed. With 100:1 ratio an increase in the 403.0 nm 3,4-benzopyrene signal was observed, due to overlap of the longer wavelength emission of pyrene with the 3,4-benzopyrene signal; if the base line method is used the net value is actually smaller and the percentage decrease is ca.22%.

3,4-Benzofluoranthene - although 3,4-benzofluoranthene has a weak fluorescence signal (roughly ten times weaker than 3,4-benzopyrene) at a 10:1 concentration ratio a percentagesuppression of 21% was observed using a concentration of 3,4-benzopyrene of $1x\ 10^{-6}$ M. Due to the very small amount of 3,4-benzofluoranthene available no other experiments were done with this compound.

Anthanthrene - anthanthrene did not produce any effect at 1:1 concentration ratio. At a 10:1 ratio ca.50% suppression was observed.

1,12-Benzoperylene - using a concentration ratio of 10:1(1,12-benzoperylene : 3,4-benzopyrene) 25% suppression was observed.

Perylene - no effect was observed at concentration ratios $<$10:1 when the concentration of 3,4-benzopyrene was 1×10^{-7}M, or with ratios $<$1000:1 when the concentration of 3,4-benzopyrene is 1×10^{-8} M.

Using a concentration of 3,4-benzopyrene of 1×10^{-7} M and with a ratio of 10:1 a suppressionof signal of 30% was observed: similar results were obtained with a 10:1 ratio, when the 3,4-benzopyrene concentrations were 1×10^{-6} or 1×10^{-5} M.

The suppression effects observed with the dibenzopyrene compounds and for 3,4-benzopyrene are probably due to an inner-filter effect on the emitted radiation and in some cases in the excitation spectrum - as in the case of the effect of pyrene or 3,4-benzofluoranthene

in the emission of 3,4-benzopyrene. Also there is the possibility of aggregation of the donor molecule caused by the introduction of an acceptor as explained by Shpol'skii and Klimova[54,55].

If collisional non-radiative transfer is present the quenching present usually obeys the Stern-Volmer relationship

$$R_O/R = 1 + k_Q \tau_F^o [Q] \qquad \text{VI - A}$$

where R_O is the fluorescence intensity without quencher, R the fluorescence intensity with quencher, k_Q is the bimolecular quenching-rate constant (normally about 10^8 M^{-1} sec^{-1}) and τ_F^o is the fluorescence lifetime for the luminescence specie in the absence of quencher, $[Q]$ is the concentration of quencher. The product $k_Q \tau_F^o$ is usually expressed as K_Q and is termed the Stern-Volmer constant. In this equation the k_Q term takes into consideration the possibility of quenching occurring due to diffusion which is alleviated by increasing in the solvent viscosity; one of the methods used to increase viscosity is by cooling the matrix to low (e.g. liquid nitrogen) temperatures. Quenching has, however, been observed in phosphorescence and a similar equation can be established[179].

For instance, the quenching of toluene phosphorescence by cis-piperylene at 77 K in an isopentane glass, according to Froehlich and Morrison[189], obeys the Stern-Volmer equation and has a Stern-Volmer constant of 942 ± 106 M^{-1}. The confirmation of the data was done by calculating the 'sphere of quenching action' (ca. 55 Å) which was higher than the typical radii for triplet energy transfer of the order of 12-15 Å. No fluorescence quenching was observed at 77 K although fluorescence quenching is observed at room temperature.

Assuming that collisional non-radiative transfer type of quenching was present, experiments were undertaken in our laboratory using 3,4-

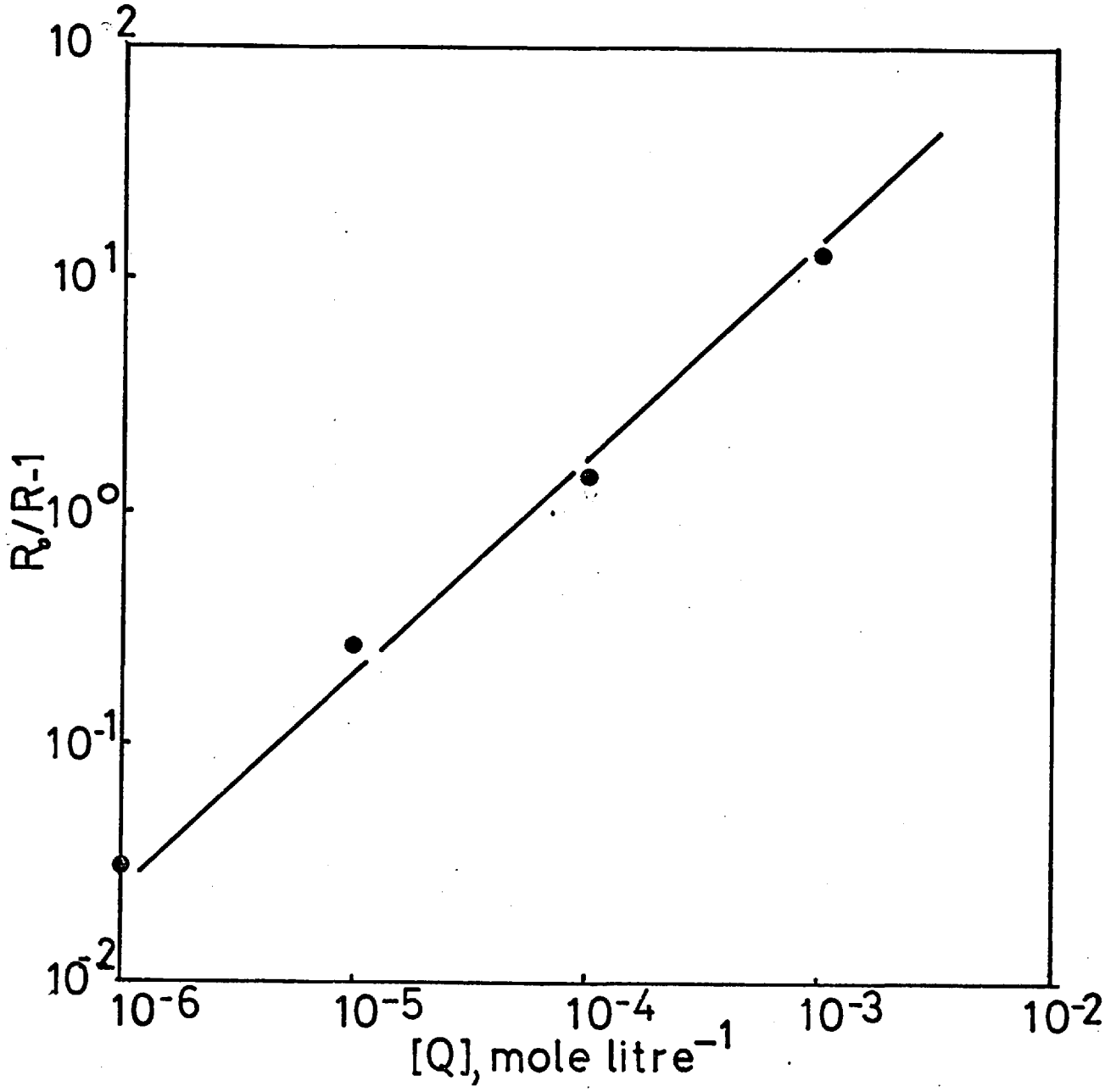


Fig. VI-1

benzopyrene as donor and perylene as acceptor. A constant concentration of 3,4-benzopyrene (1×10^{-6}M) in n-octane, was used during the experiments.

For excitation an interference filter with maximum transmission at 300 nm was employed together with a 125 W mercury vapour discharge lamp. A Stern-Volmer plot was constructed (see Fig. VI-1) where $R_O/R-1$ was plotted versus [Q]. From this plot a very high value for the Stern-Volmer constant ($K_Q = 1.4 \times 10^4$) was obtained.

If the quenching of the 3,4-benzopyrene emission is due to an inner-filter effect let us assume that absorption of the excitation light is negligible (principally in this case where perylene is better excited either with the 253.7 nm mercury line or at higher wavelength than 300 nm) and that a Lambert-Beer relationship can be established, (Formula VI-B).

$$\log \frac{R_O}{R} = \epsilon b[Q] \qquad \text{VI-B}$$

where R_O and R are the same terms as in equation VI-A, ϵ is the molar absorptivity of the compound which is producing the 'inner-filter' effect and b is the 'length of path' of the 'cell' in this case the depth of observation (see Fig. VI-2) - and [Q] the concentration of the absorbing molecule.

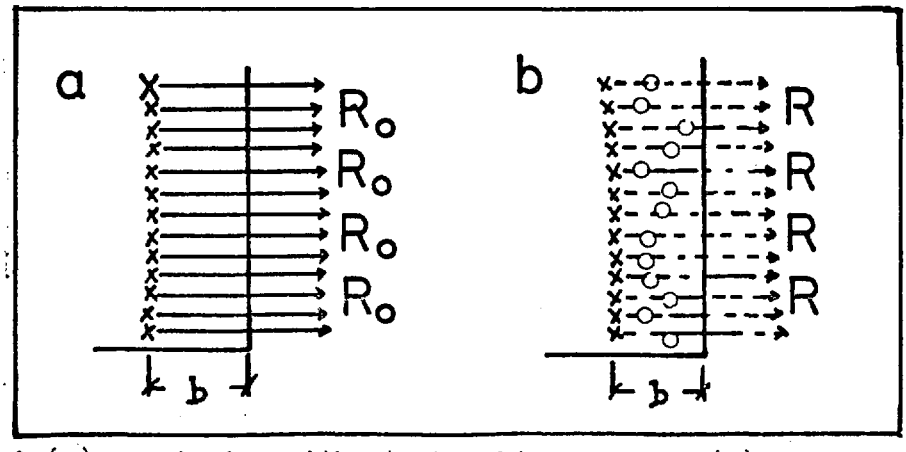


Fig. VI-2 (a) - emission without absorbing species (b) - emission with absorbing species. (X - emitting molecules of compound A; O - absorbing molecules of compound B.

Combining equations VI - A and VI - B.

$$\log \frac{R_o}{R} = \log \left\{ K_Q[Q] + 1 \right\} = \epsilon b[Q] \qquad \text{VI - C}$$

The 'false' Stern-Volmer constant can thus be related to the molar absorptivity as

$$K_Q = \frac{10^{\epsilon b[Q]} - 1}{[Q]} \qquad \text{VI - D}$$

Plotting $\log R_o/R$ *vs* $[Q]$ an average value for the ϵb product was found ($\overline{\epsilon b} = 6.1 \times 10^3$). If K_Q from the Stern-Volmer plot and one value of $[Q]$ is substituted in the equation VI - D (e.g. $[Q] = 1 \times 10^{-4}$M) a value of ϵb equals 3.8×10^3 is obtained which is an approximate value compared with $\epsilon b = 6.1 \times 10^3$.

If the same single value of $[Q] = 1 \times 10^{-4}$M is introduced in equation VI - B, the product ϵb is now equal to 3.7×10^3 which is a very similar result, suggesting that the major contribution to the high value of K_Q is due to the inner-filter effect.

This inner-filter effect is probably due to absorption of the 403.0 nm emission of 3,4-benzopyrene; perylene has absorption bands in the region between 400 - 406 nm.[165,190] Shpol'skii and Personov[190] determined the absorption spectrum of perylene in n-hexane at 77 K and avoided the use of perylene as internal standard as it was observed that the absorption bands of perylene distorted the fluorescence spectrum of 3,4-benzopyrene[115]. Narrow bands were observed, by Shpol'skii and Personov[190], at 400.70, 402.51, 403.79 and 405.75 nm in the absorption spectrum of perylene. Also, Clar[165], determined the absorption spectrum in methanol-ethanol at 103 K; the ϵ of perylene at 404.5 *nm* is *ca.* 2.0×10^4. The possibility of inner-filter action due to overlapping of the emission spectrum of 3,4-benzopyrene and the absorption spectrum

of perylene is thus quite probable.

The presence of an inner filter effect results in the necessity for analysis to either a) dilute the solution further to values of absorbance less than 0.05[168] or b) to use clean-up or pre-separation procedures before the analysis.

The possibility of avoiding the inner-filter effect by dilution is only effective if the emitting compound and the quencher have comparable quantities; the same does not apply if the amount of quencher is such that the emitting compound is a minor constituent when only a separation technique can resolve the problem.

c. Effect of light intensity

We have observed that a greater percentual suppression in the luminescence intensity was observed when the preliminary assembly (Chapter IV) was in use compared to the assembly described in Chapter V. To test if this effect was related to the source intensity, experiments were done by reducing the source intensity using an iris diaphragm between the source and the lens system. This was done by using a 1×10^{-6}M 3,4-benzopyrene solution and pyrene as quencher (1×10^{-5}M) and observing the decrease in both signals (of 3,4-benzopyrene without and with quencher) versus the decrease in light level (by closing the diaphragm). We observed, within the experimental errors an almost constant difference between the signal of the compound without quencher and the signal of the compound with quencher (Fig. VI-3). Thus the percentage suppression is bigger at low light levels.

Using perylene as quencher the same effect was observed; using a concentration of 3,4-benzopyrene of 1×10^{-6}M and of perylene of

1×10^{-5}M the following data were obtained (table VI-2).

Diaphragm Aperture (Arbitrary numbers)	0	1	2	3	4	5
Suppression (%)	9.4 ± 1	14.7 ± 2	14.3 ± 2	23.4 ± 5	$20.9\pm$	21.4 ± 5

This experiment suggests that the greater the excitation power available the less will be the effect of quenching caused by the inner filter effect.

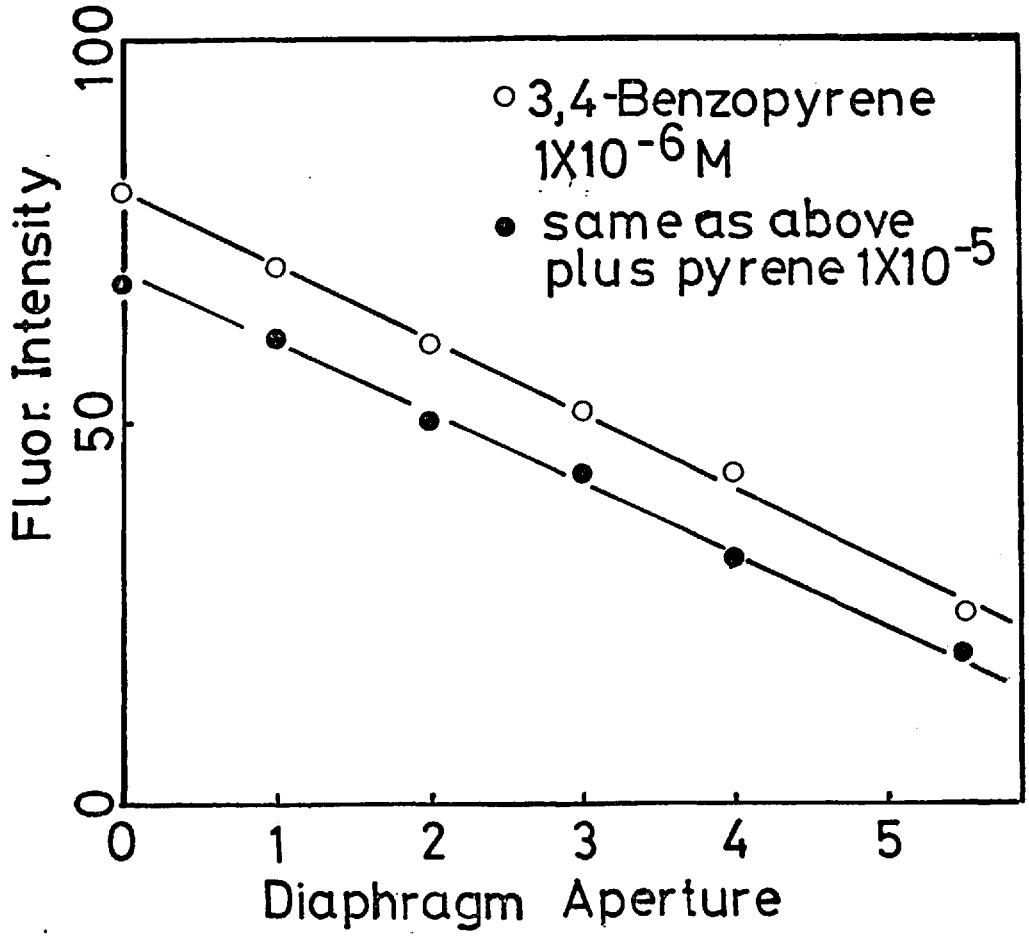


Fig. VI - 3

Chapter VII

Sample Handling Systems - The Dewar Flask and the Copper Cryostat

Introduction

The sample handling systems employed to work with quasi-linear spectra can be divided into a) - the types used for analytical purposes, where liquid nitrogen is the usual liquid coolant - owing to its low cost and wide availability and b) - the types used mostly for fundamental spectroscopic studies where the liquid coolant can be either liquid nitrogen, liquid hydrogen or even liquid helium.

For analytical applications the usual arrangement is a Dewar flask where a sample tube is introduced directly into the liquid nitrogen[118]. Quartz[124] or more frequently an ordinary glass test tube,[114-116,119,123] has been used to hold the sample (Fig. VII-1a). Dikun[114] used two concentric test tubes, the sample being frozen between the walls of the two tubes, to study the effect on the signal of the thickness of the sample layer; the only effect observed was the loss in intensity when this thin arrangement was used in comparison with the use of a test tube of 12-14 mm in diameter.

The inner diameter of the sample tube (used with the Dewar flask)is usually in the range of 7 mm to 15 mm, and the capacity ranges from 1 ml to 10 ml. The Dewar is in most cases constructed of glass. Jäger and Lugrová[111] used a specially made type of glass cell with a disc-shaped foot to ensure its attachment to the bottom of the Dewar (Fig. VII-1b). Eichhoff and Köhler[121] used a horizontal sample holder on top of a copper cold-finger (Fig. VII-1c) similar to the sample device used by Bowen and Brocklehurst[17]; Eichhoff and Köhler, to solve a problem of reproducibility, used the smallest possible volume of sample (0.06 ml).

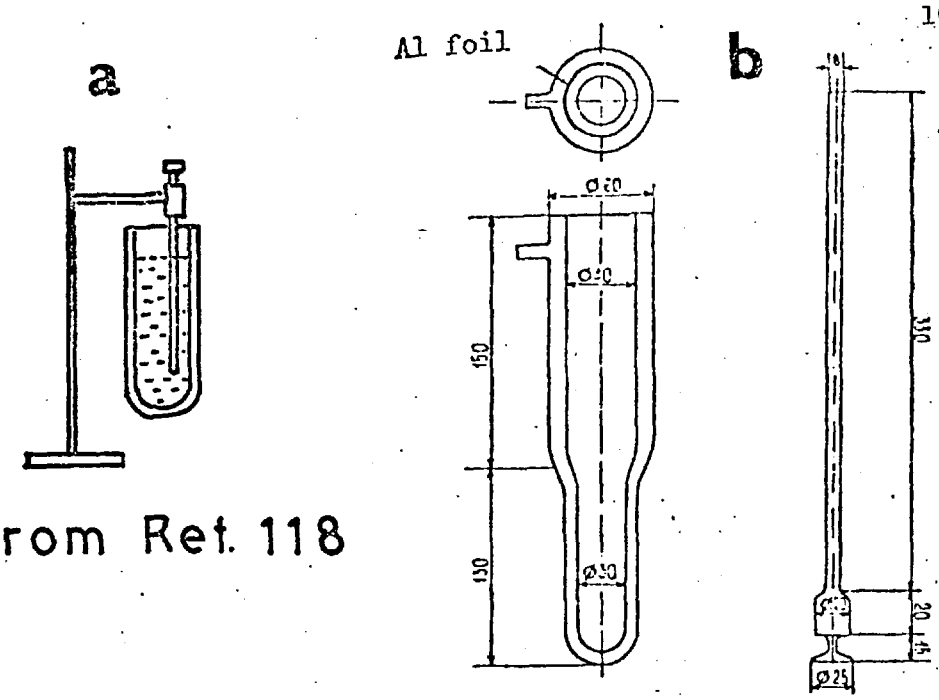
Increasing the volume a loss in resolution was observed by these workers. Also, in place of introducing the sample in the top of the cold-finger, the sample was introduced in a very small aluminium capsule (11 mm diameter and 0.03 mm thickness) but no detail was given concerning the contact established between the copper cold-finger and the aluminium capsule. The appearance of broad peaks in the spectra suggests that the sample was not at the temperature of the liquid air used as a coolant.

Muel and Lacroix[108] used two types of sample cell; one a glass tube and another a copper tube but no more details were given in the published article.

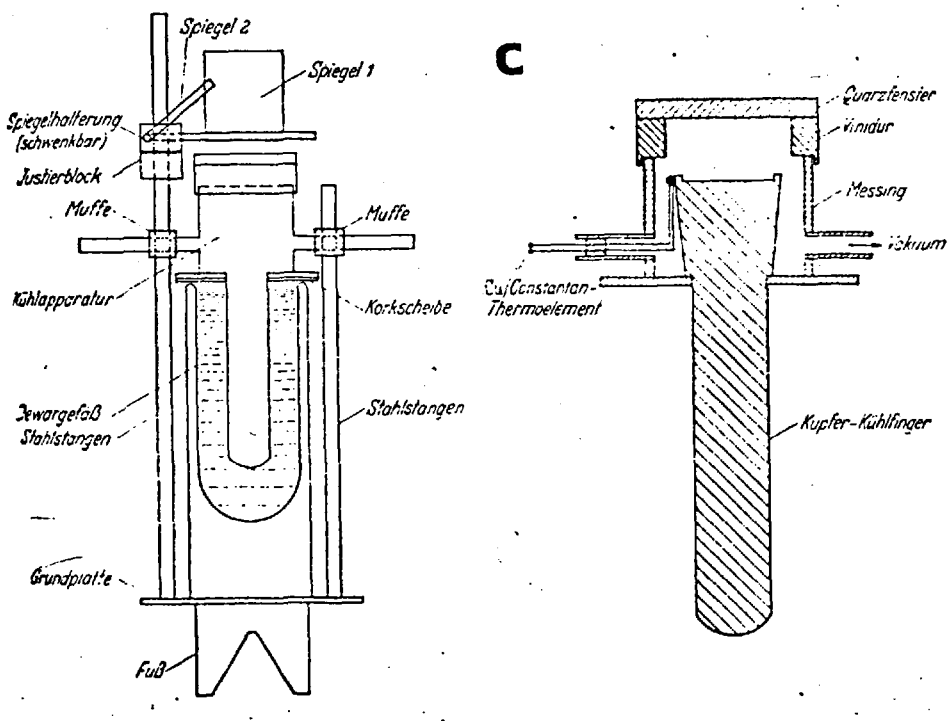
More sophisticated Dewar flasks are sometimes used by spectroscopists during the study of the Shpol'skii effect. Shpol'skii[24] described an experimental apparatus to study the effect at temperatures lower than 77 K. A paddle shaped cuvette (Fig. VII-d) was plunged into a fused silica Dewar flask and liquid hydrogen was employed as coolant. In another paper Shpol'skii and Klimova[177] described a similar Dewar using the same paddle shaped cell which was immersed in liquid helium.

Mamedov and Nasibov[178] constructed a metal cryostat (Fig. VII-2e) to work with quasi-linear luminescence spectra where temperatures lower than 77 K can be obtained by producing solid nitrogen (55 K) inside the cryostat. Two sample holders were assembled (number 3 in the figure VII-2e) at the same time and were rotated to the position of observation when necessary. An improvement in the phosphorescence spectrum of α, α'-dipyridil in n-heptane was observed at 55 K in contrast to the spectrum obtained at 77 K.

Svishchev[72] used a foam-plastic cryostat with a Teflon cell (Fig. VII-2f); the cell is, according to the author, easily assembled or dismantled when at ambient temperature. With this cell Svishchev



From Ref. 118



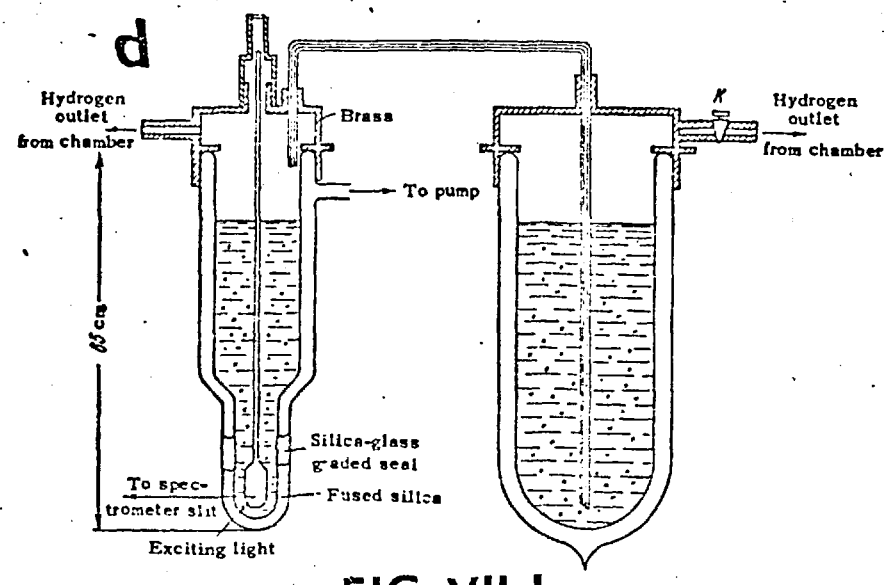


FIG. VII-1

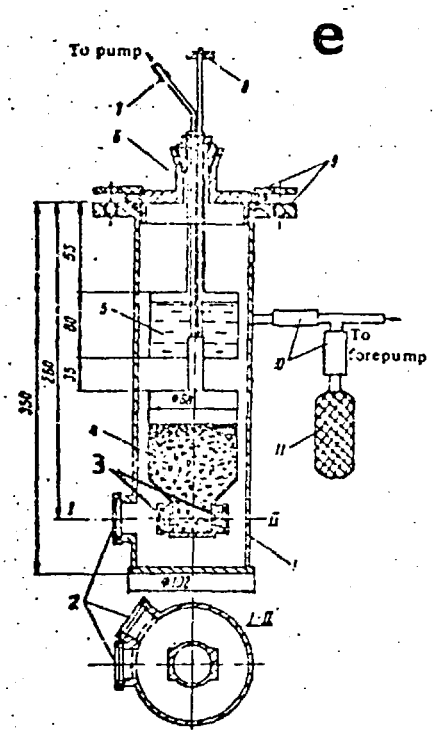
studied the quasi-linear luminescence excitation spectrum of pyrene, 3,4-benzopyrene and coronene in normal paraffins at 77 K. There is no information about the freezing rate of this type of cell, which is probably slow due to the material of which it was constructed.

Independent of the work with the quasi-linear luminescence a number of workers have used different cells, commercial or laboratory-made for other studies of low temperature luminescence (principally phosphorimetry).

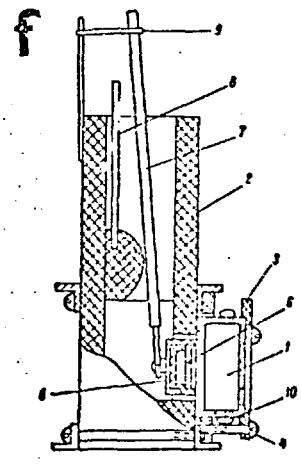
The most common cell used is of the immersion type in which a small diameter quartz tube is located inside a small quartz Dewar. This arrangement was briefly described in Chapter IV and will be further discussed in the present Chapter. Other similar arrangements (Fig. VII-2g) have been described by Winefordner, Schulman and O'Haver[179]. Parker[158] has described another more elaborate assembly including one device for measuring the luminescence of thin layers of rigid solutions at 77 K by frontal illumination (Fig. VII-2h). A small tray (T) with a rectangular depression (20 x 30 and 3 mm deep) where the sample is introduced, slides in a groove into the front face of a copper block and makes close contact with the surface of the latter. This block has contact with a copper rod which is then immersed in liquid nitrogen.

Kirkbright, Mayne and West[180] described a Dewar tube assembly (Fig. VII-2i) in which some of the problems created with the immersion type of Dewar cell were eliminated and more reproducible sample cell positioning and lower noise levels were obtained at low temperature. A modification was also made in this assembly for work with EPA[181] when, due to the temperature of the system, EPA was sufficiently mobile to be pumped through the cell.

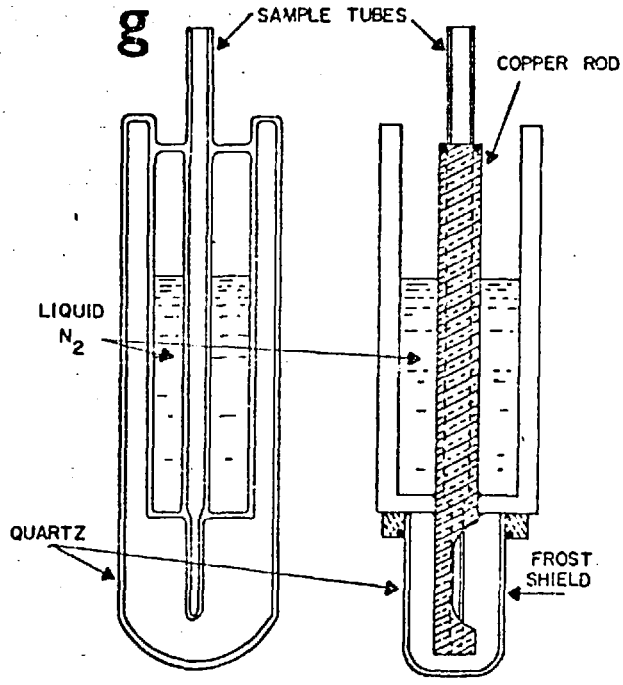
McHard and Winefordner[182] have described the construction and use

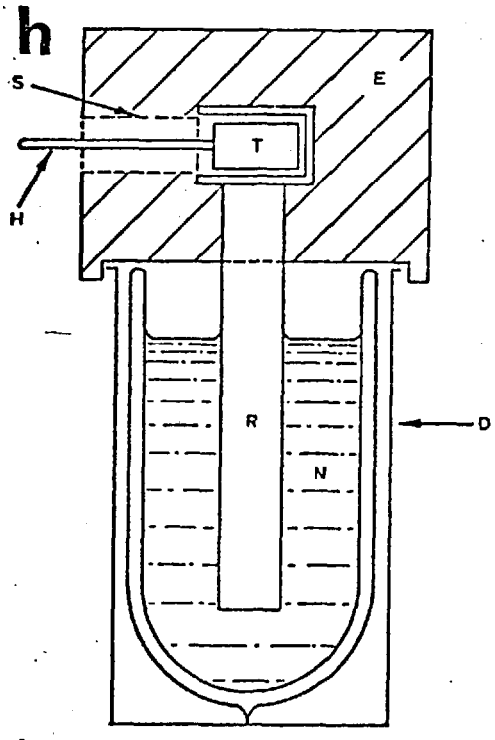


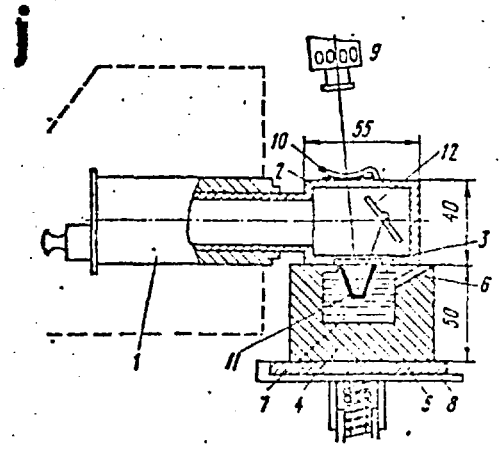
Cryostat for luminescence investigations: 1) case; 2) windows for excitation and observation of sample; 3) cuvettes; 4) solid-nitrogen tank; 5) liquid-nitrogen tank;



Foam-plastic cryostat.







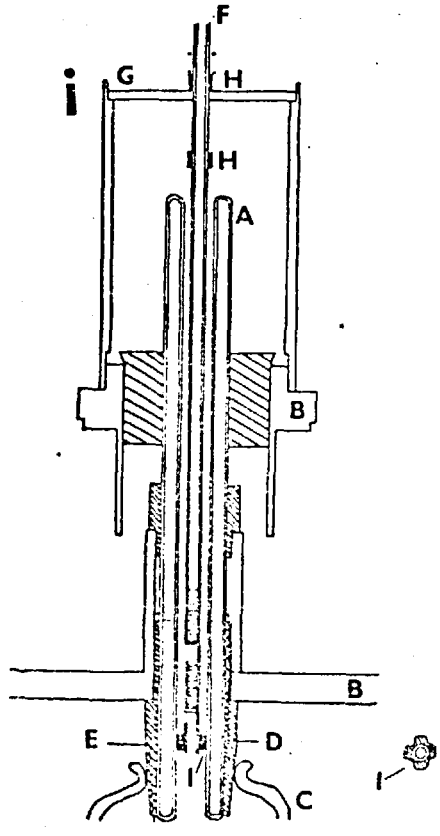


FIG. VII-2

of a windowless, front surface illumination cell for low temperatures fluorescence and phosphorescence measurements. The cell can be used with solids, pastes and viscous solutions. The temperature of the sample was, when the equilibrium was attained, 133 K. The windowless microcell was assembled in the top of a cold finger, which was immersed in liquid nitrogen. Cold nitrogen vapour was also directed into the sample housing to avoid condensation of atmospheric water vapour and as long as the residence time of the sample cell in the housing is less than 20 minutes, condensation was not troublesome.

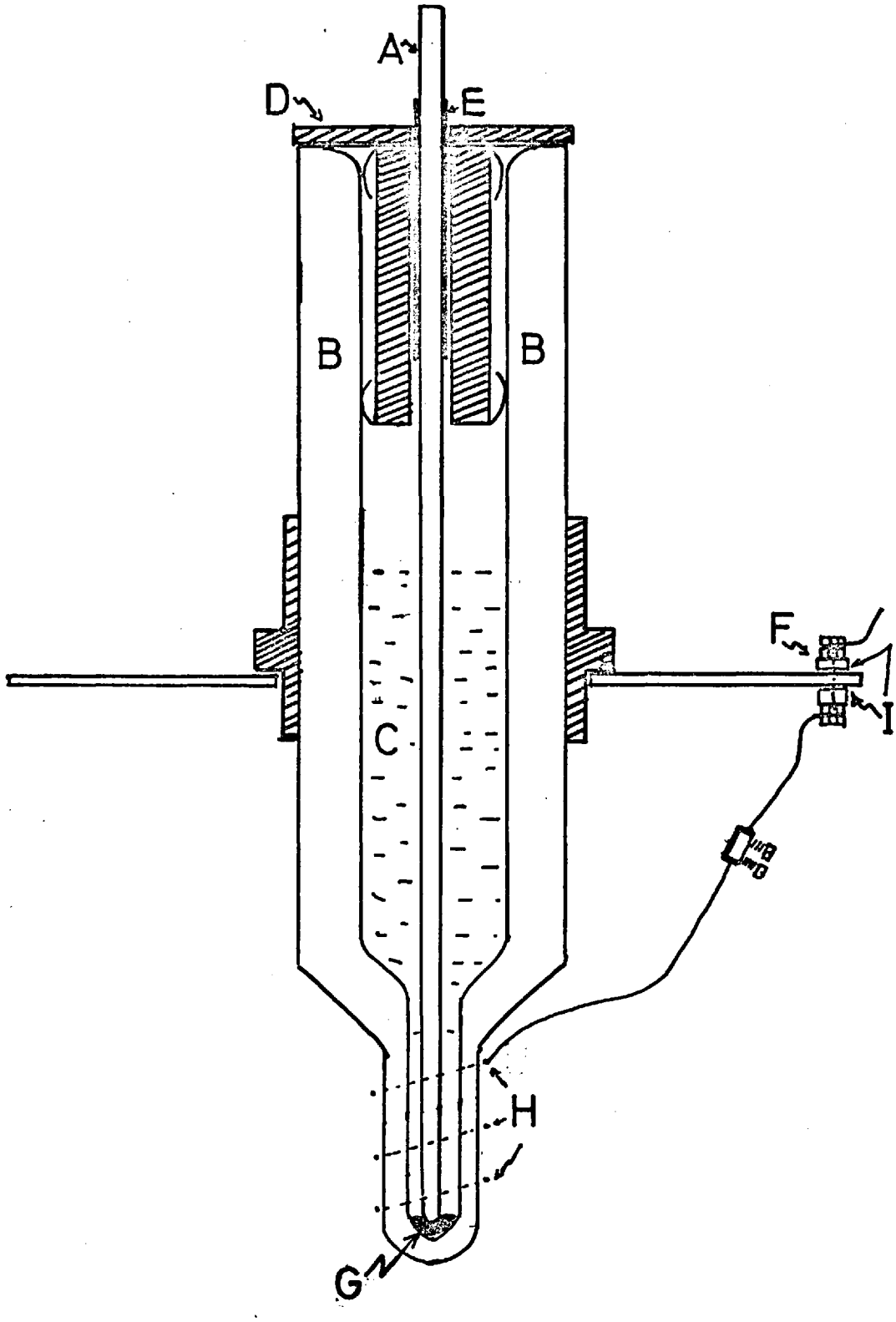
For work with inorganic solutions Vasil'ev, Solov'ev and Bozhevol'nov[183] have recently proposed a sample cell attachment, made with a metal crucible (number 11 in Fig. VII-2j) in contact with liquid nitrogen: the frozen solution is illuminated from the top and a mirror guides the signal (and scatter) to the monochromator.

Evaluation of the Dewar Flask Sample Cell System

The Dewar flask system employed in our experiments is the same as that commercially available (American Instrument Co.) except that in our laboratory it has been used with a silica tube (Spectrosil) sample cell of ca. 200 mm length and 3 mm internal diameter and 0.6 - 1.0 mm wall thickness. Liquid nitrogen was used in all experiments. The volume of sample solution used is 0.3 - 0.5 ml (Fig. VII-3).

With this cell assembly the process of freezing is very fast and the final temperature is stable at 77 K. The system, however, suffers from some disadvantages. First is the problem of the reproducibility with which the tube may be positioned. This problem has been solved

Fig. VII-3



A. Sample tube. B. Dewar vessel. C. Liquid Nitrogen. D. Support. E. Plastic sleeve. F. Connection. G. Plastic "foot". H. Nichrome coil. I. Heat insulator.

previously by the use of a different Dewar and cell assembly designed by Kirkbright, Mayne and West[180] already cited and by Hollified and Winefordner[184] who developed a rotating sample cell assembly to minimise the error and at the same time average out the optical inhomogeneities in the sample tubes.

Other problems are caused by the condensation of atmospheric water vapour on the Dewar flask and in the liquid nitrogen. Noise is also introduced due to the constant boiling of the liquid nitrogen in the optical path. In contrast to the low resolution spectrofluorimeter assembly the time required to obtain a complete spectrum is longer (15 - 20 minutes) and in consequence the problem of condensation is of critical importance. Attempts were therefore made to solve the problem by the use of dry nitrogen; however with the long residence time needed to obtain a spectrum a better solution was found via the use of a coil of Nichrome wire (2.18 $\Omega\ m^{-1}$) placed around the outer wall of the Dewar flask (H). The wire was heated by an applied voltage of ca. 10 V a.c. using a rheostat between the coil and the electrical mains supply.

An additional problem results from water vapour condensation in the liquid nitrogen with consequent formation of snow particles; this was minimised by not allowing the liquid nitrogen level to drop inside the optical path as most of the snow particles float on the surface of the boiling liquid nitrogen.

Due to the small capacity of the Dewar flask (ca. 20 ml) constant refilling was necessary with consequent interruption of the scan. Another design with larger capacity (ca. 50 ml) has been made and has been constructed and is now in process of evaluation.

The positioning problem was improved by the use of a plastic sleeve (E) in contact with the perforated support (D) of the Dewar flask (B). Also a plastic foot (G) was used to hold the base of the silica sample tube (G). Before the measurements the tube was manually rotated until the maximum signal was obtained and a much better situation was obtained in comparison to the usual way of attempting to optimise the position of the tube before introduction to the spectrometer; similar procedure of maximum signal was used by McGlynn, Neely and Neely[185].

In some experiments (constant time and constant change display systems; see Chapter IX), a small motor adapted from a mini-stirrer (Eletrothermal Ltd.) was used to rotate the sample tube and reproduce the method used by Hollifield and Winefordner[184]. However, although the motor introduced a better signal reproducibility the vibration and the noise were unacceptable and the system was abandoned. Zweidinger and Winefordner[186] now use a commercial nuclear magnetic resonance sample tube spinner, rather than their early laboratory-made system.

As observed before although the Dewar flask system has the advantages of fast sample cooling and a final, quite stable and well defined temperature, being the disadvantages the noise introduced by the convective motion and bubbling of the coolant and the exact positioning.

The modifications proposed in figure VII-2g eliminate these problems; however in the first system (left Dewar flask) the sample temperature is uncertain and inhomogeneous and there are problems involved in changing the sample;[179] in the other system (right Dewar flask) with the copper rod the problem is the fogging in the frost shield as the system cannot be evacuated[179]. The system proposed by Eichhoff and Köhler[121] or by Vasil'ev, Solov'ev and Bozhevol'nov relies on the use of a mirror or prism to introduce the luminescence signal to the mono-

chromator.

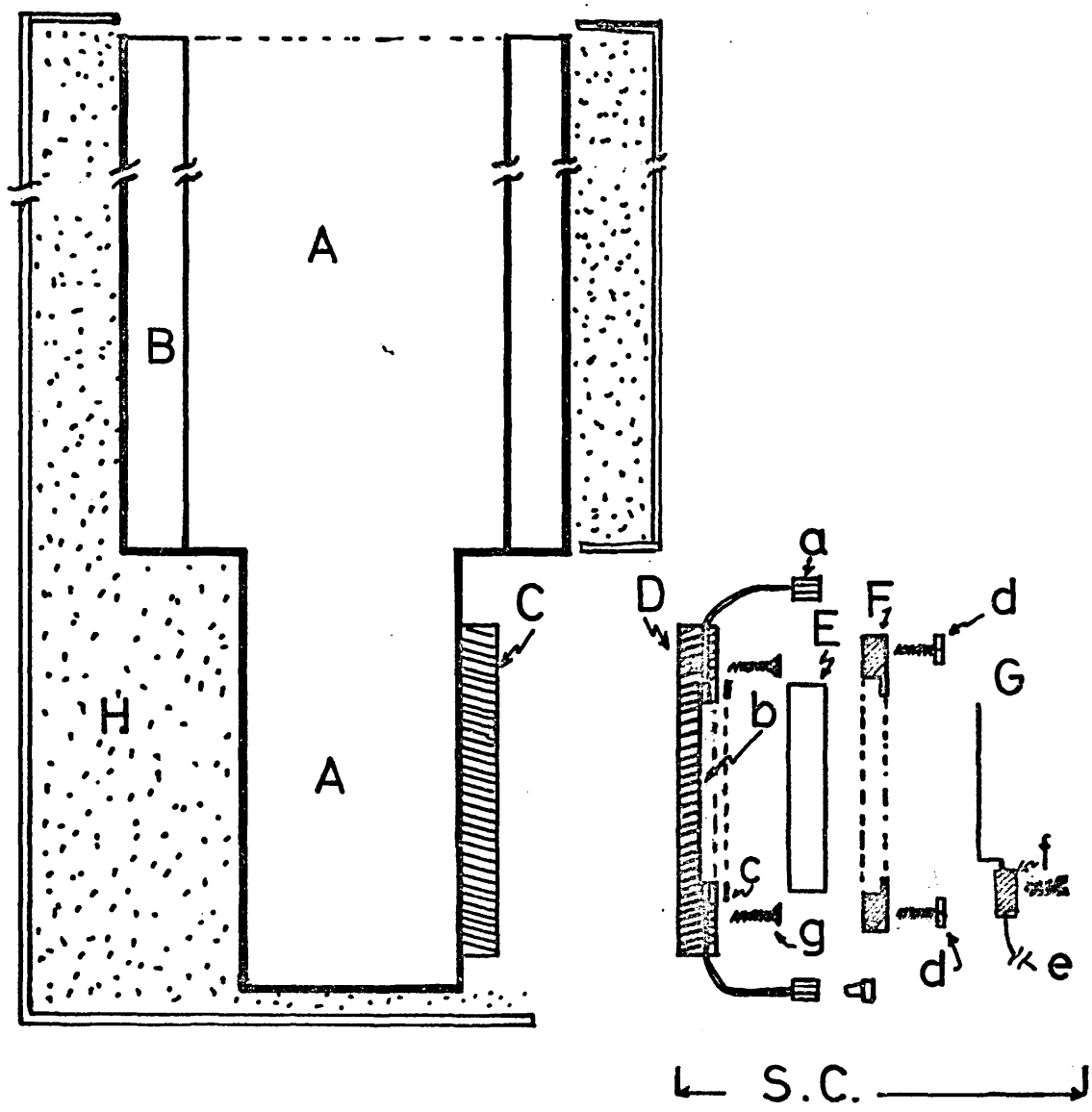
A system similar to those proposed by Parker[158] and Svishchev[72] without the inconvenience of the latter, where to change the sample it is necessary to warm the whole system, was then constructed in our laboratory. In the particular case of compounds with biological activity e.g. some of the carcinogenic polycyclic aromatic hydrocarbons, aflatoxins, etc. the system must be of practical use without the possibility of spillage or contamination. The system also must have a definite temperature and a reproducible and not extremely slow cooling rate.

Construction of the Copper Cryostat

Figure VII-4 shows the constructed assembly. The copper reservoir (A) into which the liquid nitrogen is introduced was surrounded with expanded polystyrene (H) as heat insulator. Also in the upper end of the reservoir a vacuum space (B) was provided to decrease the heat transmission. At the lower end is a flat, vertical, surface (C) to which the sample cell (D,E,F,G sections are together during the experiments), is attached by the use of four copper screws (g). The copper cell (D) which forms a sample compartment with a capacity of ca. 0.5 ml. Two small diameter tubes (1.58 mm) which end in a syringe type connections (a) provide for filling and flushing the cell. The sample compartments extends, slightly, outside of the excitation area, as most of the solvents used contract when frozen (see table VII-1).

The sample compartment was isolated from the ambient temperature by a quartz microcell (E) which was evacuated several times and filled to low pressure (10 torr) with dry nitrogen. The quartz microcell was then sealed to create a vacuum chamber. This vacuum chamber was used as

Fig. VII-4



A. Liquid Nitrogen Reservoir. B. Vacuum Space
C. Cell Copper-Support Plate. D. Copper Cell.
E. Quartz Vacuum Chamber. F. Clamp.
G. Nichrome Wire Turn. H. Polystyrene Foam.
a. Syringe Connection b. Sample Space.
c. Washer. d. Teflon Screws. e. Wire Terminal
f. Asbestos Support. g. Copper screw. S.C. Sample cell.

TABLE VII-1

Volume Contraction of Matrices (in percentage)

Ethanol	17[1]	n-Hexane	20
EPA	30[1]	n-Heptane	17
THF	18	n-Octane	11.5
n-Pentane	20	n-Decane	11.0

(1 - Produces glassy matrix when frozen)

a window and a thin rubber washer (c), was used to seal the system against the copper cell wall. Lead, indium, Teflon and polythene gaskets were also tested but in these cases the pressure used to seal the system was such that the vacuum chamber breaks during the cycles of freezing and warming. The vacuum chamber was tightened in position using an aluminium front plate (F) and nylon screws (d). These screws are slightly flexible at room temperature and have the advantage of being poor conductors. Care was exercised not to over-tighten the nylon screws as this again caused cracking of the vacuum chamber.

The front window of the chamber was defrosted using a single turn of Nichrome (G) wire (2.18 $\Omega\ m^{-1}$) which was heated using the mains supply at 2-3 V a.c. supplied across a rheostat. Attempts were also made to use a single quartz window but when defrosted with the heated coil the frozen sample then became warm. Attempts to use a strong flow of dry nitrogen as an alternative failed to prevent frost appearing in

the window after a short time. Plate VIII-1 shows a photograph of the Copper cell assembled in the instrument.

Procedure

Dewar - Silica Tube

0.3 - 0.5 ml of the solution to be analysed was introduced into the silica sample tube using a small Pasteur pipette provided with a rubber bulb. The tube was plunged into the liquid nitrogen already present in the Dewar flask. After the boiling ceased the Dewar and silica tube were placed in the cell compartment. The heating coil was switched on and after the exterior wall had defrosted the scan was started. At the end of the analysis it was possible to remove the silica-tube maintaining the Dewar flask in position, and after the frozen solution had melted the solution could be removed by gently tapping the tube. The silica tube was washed with a solvent, with the new solution and finally filled again for reuse.

Copper Cryostat

In the sample cell, being disconnected from the support plate and at room temperature, the solution to be analysed was introduced, using a 1 ml syringe, through the bottom syringe connection. To fill, the cell was maintained slightly tilted to avoid any bubbles being trapped in the optical path. The cell was filled until the liquid appeared in the outlet tube. The syringe was removed and the bottom syringe inlet was stopped with a small Teflon stopper. The cell was then partially submerged in a liquid nitrogen bath, still in a tilted position to avoid

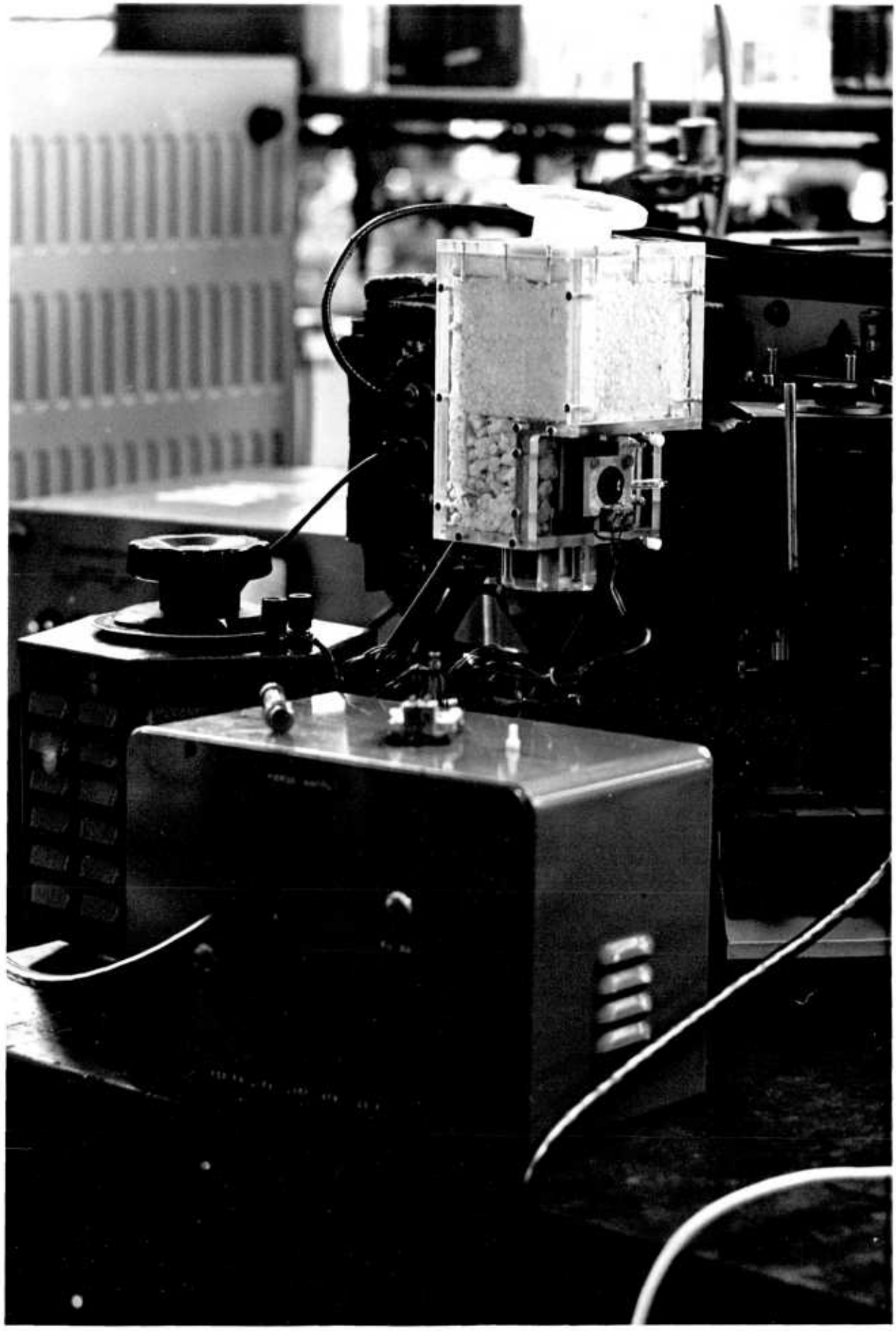


Plate VII-1

the appearance of an empty volume in the front of the window due to the contraction of volume of the frozen matrix. Care was exercised to avoid contact of the liquid nitrogen with the silica microcell, at this stage, as the cell may crack.

The freezing process was undertaken with the cell and the holding screws already in position: this avoided future problems of lack of fitting due to the different temperature and consequent different dimensions of the holes in the support plate (c) in the liquid nitrogen reservoir.

After boiling subsided and when the cell support plate was also at liquid nitrogen temperature (and had been cleaned of any snow deposit with a paper tissue) the cell was rapidly transferred and tightly connected to the support plate by the use of the four available screws. Finally the heating coil was applied to defrost the external window.

At the end of the analysis the cell was disconnected from the plate, and after the frozen solution had melted the cell was washed with the solvent using a syringe and a new solution was introduced for the next experiment.

Care and Maintainence of the Copper Cell

Periodical cleaning of both cell and support plate contact surface with fine emery paper and a paper tissue was necessary. When necessary the rubber gasket (c) was changed as, due to the cycles of freezing and warming, small cracks eventually appear in this.

Freezing Rate and Final Temperature

As already described in the Chapter II of this thesis the freezing rate plays an important role in the shape and intensity observed for quasi-linear luminescence spectra.

To compare the freezing rate of the conventional silica-tube-Dewar flask and our cryostat assembly, an arrangement was made using two copper-Constantan thermocouples; the EMF was measured using a Servoscribe recorder (Model RE 511.20). The thermocouples were tested and the measurements were calibrated using liquid nitrogen, ice and solid carbon dioxide and the EMF was related to temperature using the appropriate table from Ref. 187.

It is interesting to note that, based on an observation made by Personov[115] - switching from rapid freezing to slow freezing the intensity ratio between the long wavelength and the short wavelength of the doublet of coronene in n-hexane increases. A qualitative estimation of the freezing rate by the comparison of this ratio is possible, although there is no direct correlation at first sight between the freezing rates observed and the intensity ratios (see table VII-2).

The measuring thermocouple was introduced into the solvent either when inside the silica tube or in the sample compartment. The reference thermocouple was introduced into a Dewar containing melting ice.

The freezing rate of the copper cell was much slower than the silica tube which is very rapid. Highest freezing rate was obtained by Cohen and West,[188] by finely nebulizing the solution and spraying the mist directly on to the surface of liquid nitrogen. In their work (which was not related with quasi-linear luminescence) Cohen and West estimate this freezing rate to be 10^4 °.sec^{-1}. This process is not,

however, of practical use in our case, due to the toxic character of the compounds in use.

The freezing rate of the copper cell is, however, still fast; Mishina and Nakhimovskaya[69] considered 'slow' cooling to be 10^{-2} °. sec^{-1} ($0.6°.min.^{-1}$) and an 'average' rate of cooling 0.5 to 1.0°. sec^{-1} ($30 - 60°. min.^{-1}$). Table VII-2 shows the freezing rate of different silica tube and the copper cell and also the intensity ratio of the doublet emission of coronene (445.15 and 443.44 nm) in n-hexane.

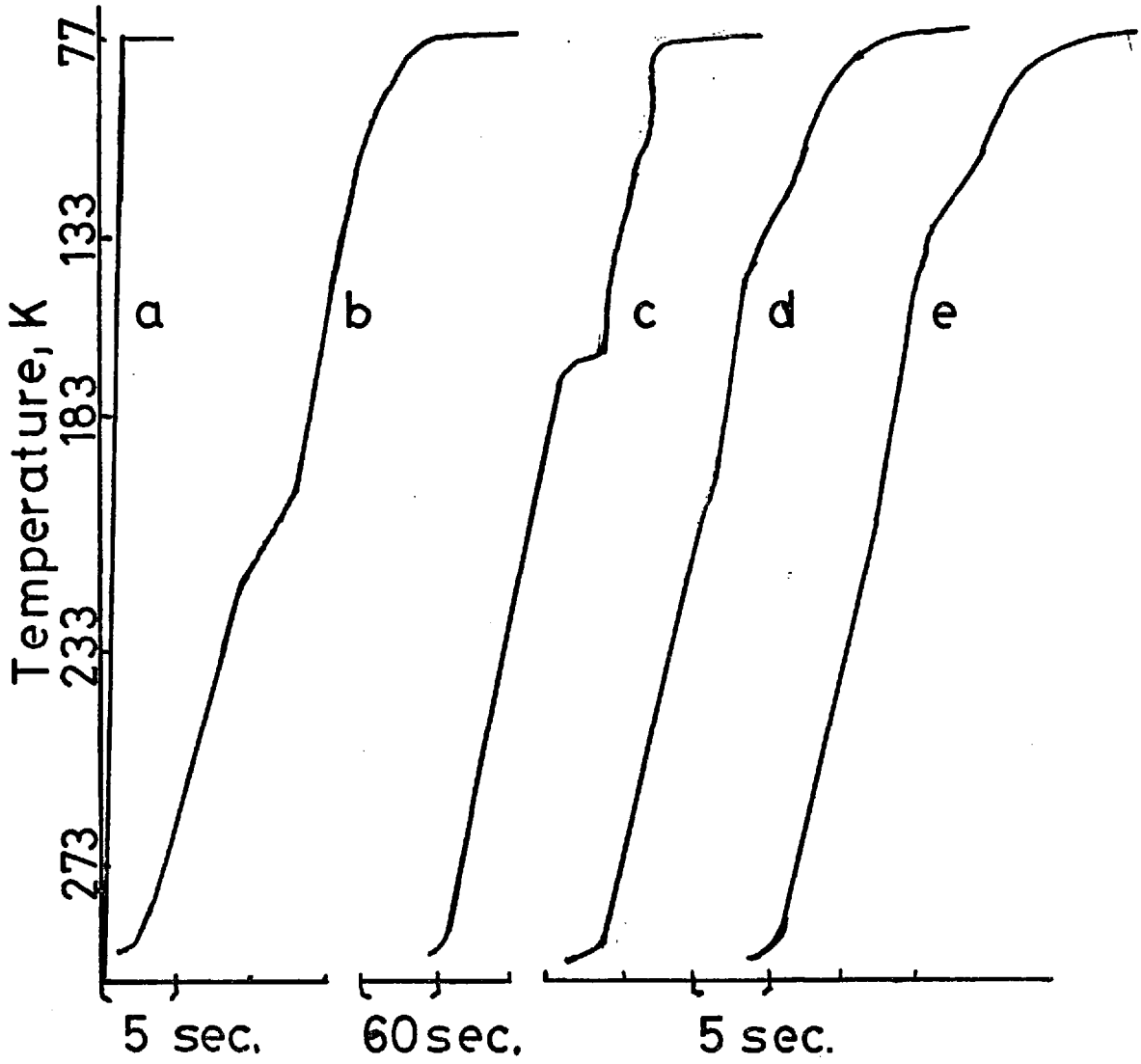
TABLE VII-2

Sample Device		$I_{445.15}/I_{443.44}$	Rate of Freezing °. min^{-1}
Silica Tube	Thin wall (0.6 mm)	3.28 ± 0.2	720
	Thick wall (1.0 mm)	3.75 ± 0.2	540
Copper Cell	-	4.05 ± 0.05	75

The final temperature after freezing the silica tube was 77 K; the copper cell after freezing was also 77 K when in the liquid nitrogen bath and 80 K after being assembled in its final position.

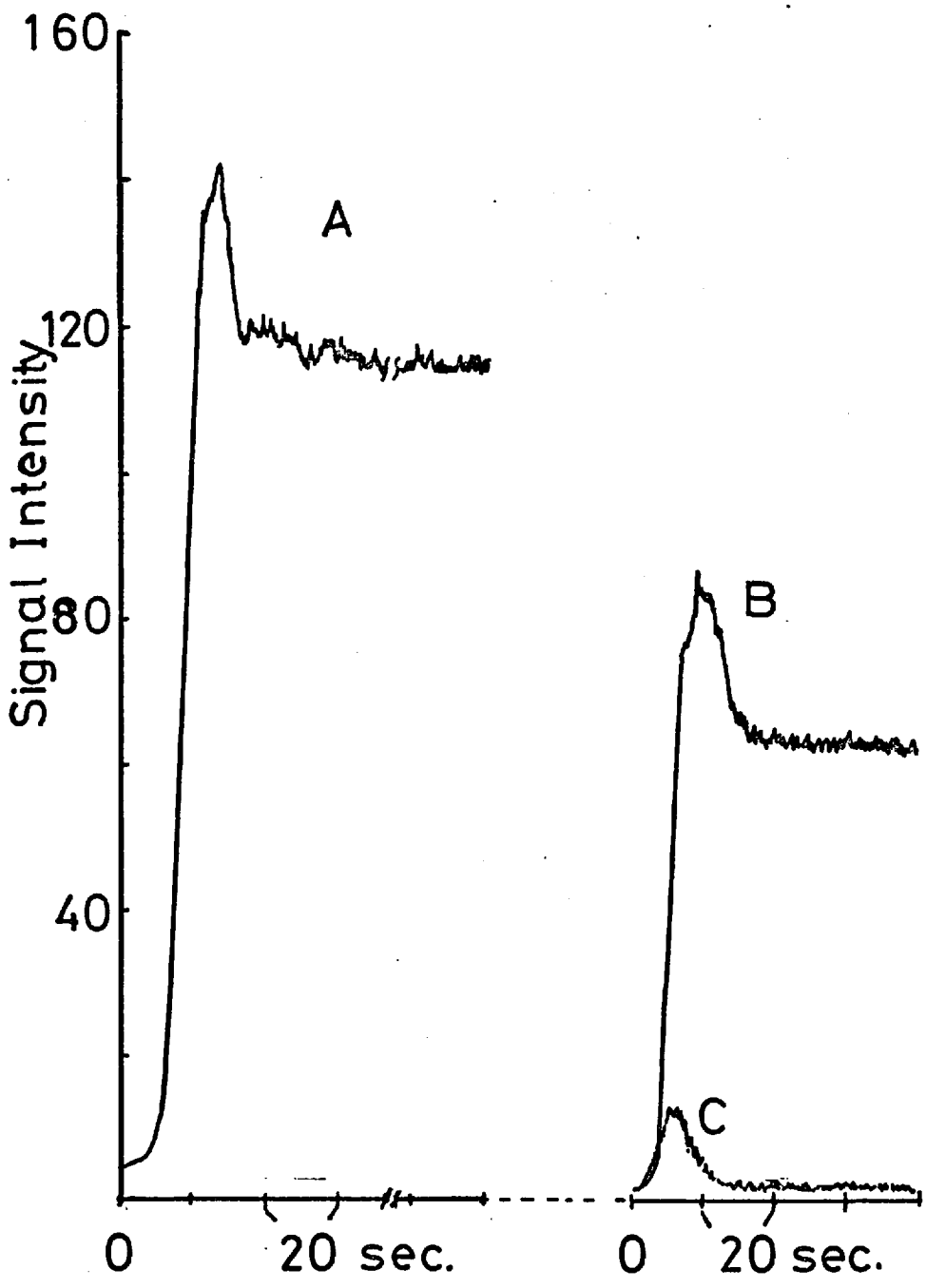
Freezing Rate Curves and Scatter Radiation

During the determination of rate of freezing the curve of thermocouple reading (mV) vs time was automatically recorded (Fig. VII-5). In



a. Termocouple introduced in liquid nitrogen.
b. Silica tube with n-octane.
c. Copper cell with n-hexane.
d. Silica tube with THF.
e. " " " n-pentane

Fig. VII-5



A. 3,4-Benzopyrene ($1X10^{-6}M$) at 403.0 nm

B. n-octane at 404.65 nm (mercury scatter)

C. EPA at 404.65 nm.

Fig. VII-6

all cases it was possible to observe an inflection point where probably crystallization of the solvent takes place. In some cases such as n-pentane, n-hexane, n-heptane and THF it is possible to observe a second inflection point. Visual observation of the freezing process shows that when crystallization occurs a volume contraction takes place, as already determined. After a very short time, however, a very small expansion is observed, suggesting another modification in frozen polycrystalline matrix. Other crystalline modifications have been reported in some of these solvents as was previously described in Chapter II; it is possible that some of these modifications may explain the second inflection point; however, only a careful study together with crystallographic data will clear such point.

Another effect that is observed during the freezing process is the expected signal increase, however, an enhancement is first observed in the signal and this latter decreases and then remains constant (Fig. VII-6A). The same effect was observed with a mercury line scatter (6B,C) in different solvents, even in EPA but to a lesser extent, showing that this effect is due to change in the scatter properties of the silica-tube-matrix during the freezing process.

Results

A comparison of both cell systems was made using frontal illumination (45^{o}) and using the monochromator refered to in Chapter V. (Rank-Hilger Monospek 1000).

The figure VII-7 shows the spectrum of 3,4-benzopyrene in n-octane, at 77 K, in both cells, at medium sensitivity

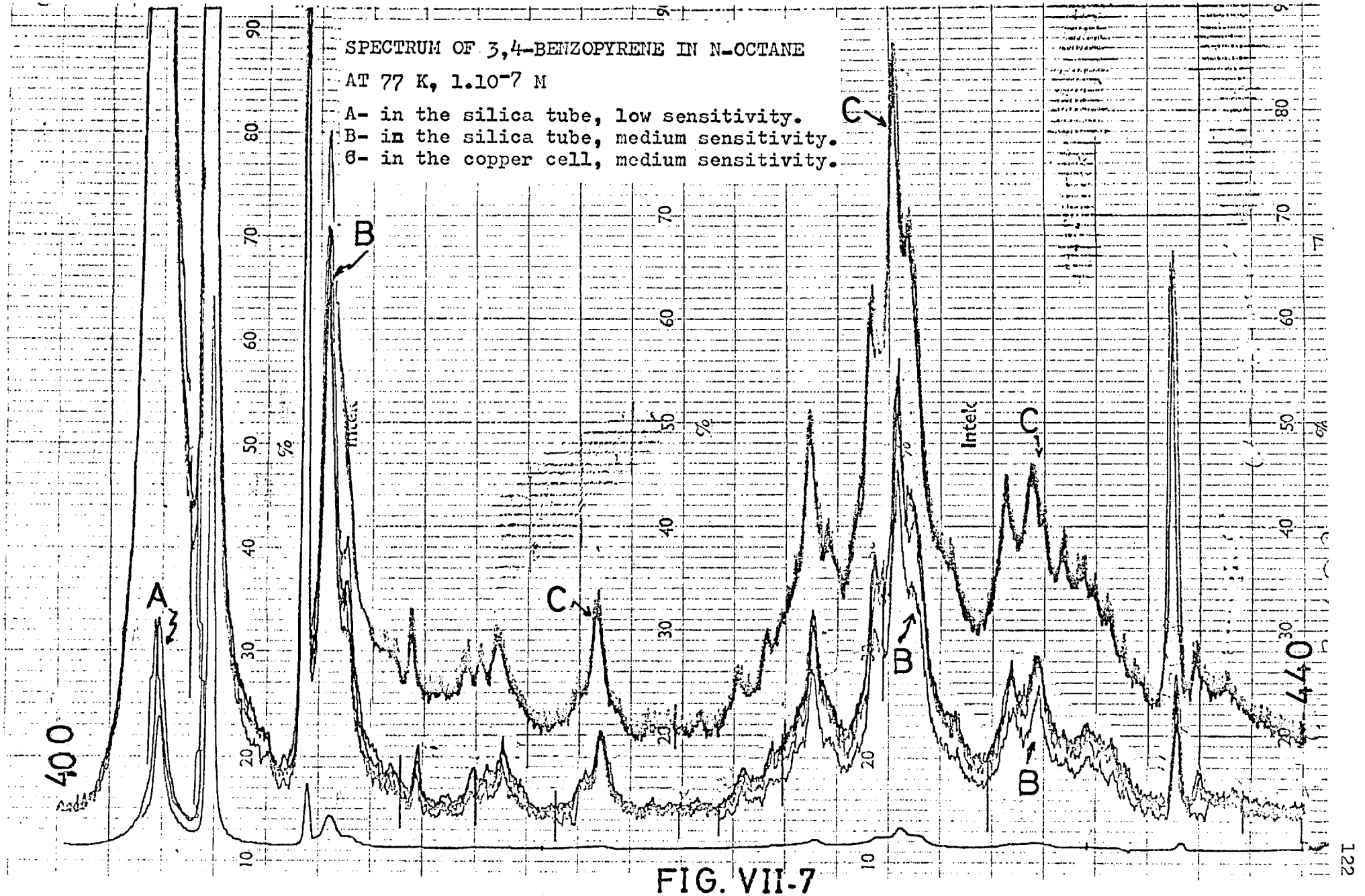

SPECTRUM OF 3,4-BENZOPYRENE IN N-OCTANE
AT 77 K, 1.10⁻⁷ M
A- in the silica tube, low sensitivity.
B- im the silica tube, medium sensitivity.
C- in the copper cell, medium sensitivity.
A
B
C
Intens.
%
400
440

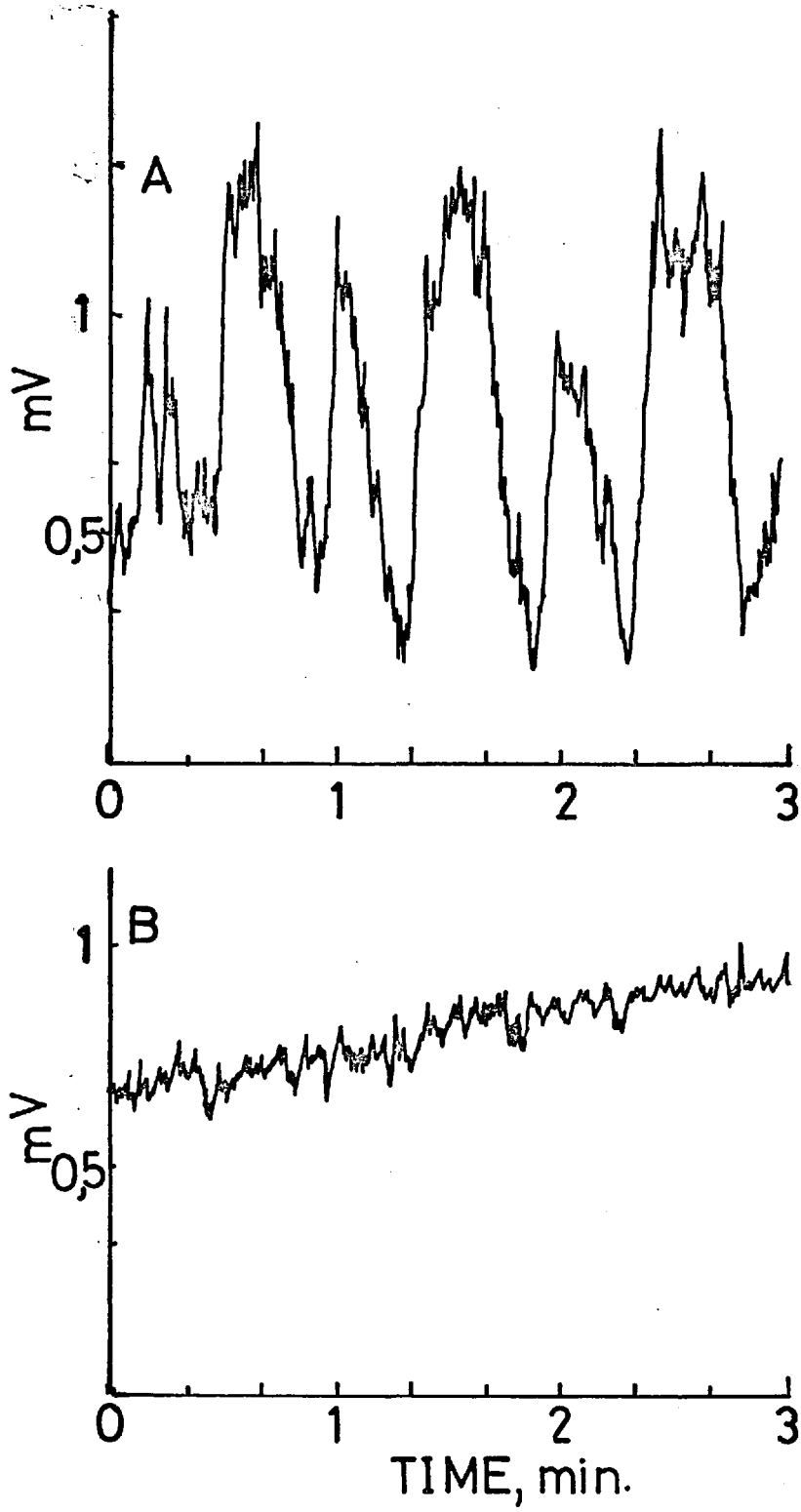
FIG. VII-7

obtained in several experiments. Good signals and quite good reproducibility is observed. The relative standard deviation of the measurements for the copper cell in comparison with the silica tube can be seen in table VII-3. A substantial increase in precision is obtained with the copper cryostat as can be seen from the lower relative standard deviation in comparison with the silica tube cell assembly.

TABLE VII-3

	Silica Tube	Copper Cell
Coronene (in n-hexane, 2.5×10^{-5} M)	9.7%	1.5%
Coronene (in n-hexane, 2.5×10^{-7} M)	12%	2.5%
3,4-Benzopyrene (in n-octane, 1.0×10^{-7} M)	4%	1.3%
3,4-Benzopyrene (in n-octane, 1.0×10^{-9} M)	18%	11%

The limits of detection are similar. Although the noise is smaller with the copper cell, due to the construction of the copper cell the area of frozen solution excited is smaller in comparison with the silica tube-Dewar arrangement — the signal strength is smaller — the limit of detection with the copper cell is consequently similar (Table VII-4)



A - Mercury lamp (MBW/U 125W) scatter at 400 nm - aged lamp, ~50 hours.
B - Same as A, but new lamp.

Fig. VII-8

Table VII-4

	Silica Tube	Copper Cell
3,4-Benzopyrene (in n-octane)	3×10^{-10} M	5×10^{-10} M
Noise, at 400.0 nm (mV)	ca. 0.18	ca. 0.04
Background, at 400.0 mm (mV)	ca. 0.5	ca. 0.5

The background is the same in both cases. The noise observed with the copper cell is not a function of the cell, but a function of the noise originated from the excitation source and from the detection system. In particular the mercury vapour discharge lamp (Philips MBW/U) used in most of the experiments, the noise increases with the age. Figure VII-8 shows a typical noise and drift at 400.0 mn at higher sensitivity with a MBW/U 125 W mercury source with nearly 50 hours of work in contrast with a new source of the same type. With this type of fluctuation and near the limit of detection the fluctuation can be as large as 50%, and also in some instances an almost total disappearance of signal may be observed due to drift during measurement.

Conclusions

The copper cell assembly shows a considerable improvement of precision in contrast to the silica tube assembly. Higher amplification sensitivity can be used during the scans and repetition of spectra can be done as many times as needed; weak peaks and shoulders can be

confirmed. For integration systems this arrangement is much more convenient as the addition of liquid nitrogen can be made with minimum perturbation of the measurement in progress, as the cell is maintained kinematically aligned. The flicker noise introduced due to the liquid nitrogen bubbling is removed; liquid nitrogen containing snow particles can be used as well as these are not in the optical path. Using a source with a better stabilization system the noise will be minimal and the detection limits will be considerably lowered. The sample volume can be easily reduced if necessary as in the case of a point source such a laser is used and the absolute detection limit will be undoubtedly smaller. Temperature studies (higher than 77 K) can easily be done in the future by introducing a heating element in the cell body.

The copper cryostat cell has been used in our recent qualitative and quantitative applications studies, quenching studies and also using integration systems and the photon counting technique of detection when it was possible to observe all of the advantages described above.

Chapter VIII

Tetrahydrofuran as a Matrix for the Shpol'skii Effect

Introduction

To produce a narrow luminescence spectrum in the condensed phase or, in particular, to produce a quasi-linear luminescence spectrum, apart from decreasing the temperature of the matrix, the matrix itself must satisfy several requirements: 1 - it must, as far as possible, interact weakly with the excited molecules; 2 - the molecules must diffuse, during cooling, into it without deformation so that they can be orientated precisely in determined positions, and 3 - it must be transparent in the region where the investigated substance absorbs or emits; in other words no inner-filter effect from the solvent can be present[127].

In the special case of introduction of fluorescent molecules into a crystal lattice, conditions of isomorphism must be also obtained. This creates another difficulty as the matrix not only must obey the conditions of 1 - 3 above but also must have a similarity in chemical structure and in the crystal lattice. Such organic mixed crystal conditions have been found in several studies and some have already been quoted in Chapter II: triphenylmethyl in triphenylamine[20] at temperatures of 77 and 4 K, stilbene in tolane and stilbene in dibenzyl at 20.4 K[192] and naphthalene in durene[21] when narrowing of the fluorescence bands to lines have been obtained in all cases.

On the contrary the solvents usually employed for the observation of the Shpol'skii effect - the n-paraffins - are not restricted by the conditions of isomorphism but belong to a completely different group of compounds in comparison with the investigated guest (analyte) molecule.

The various n-paraffins differ by the number of - CH_2- groups and represent a large selection of molecules with different chain lengths, readily crystallizable, transparent when pure up to the far ultraviolet region and are chemically neutral[44].

The properties of being chemical inert and optically transparent are not themselves, however, sufficient; spectra of various compounds (including benzene) in frozen inert gases as argon, kripton and xenon at low temperatures (4.2 K) have been obtained by Bolotnikova[35,36]; but the spectra obtained were considerably more diffuse than those in n-paraffin solutions at the same temperature. Also, the spectrum of benzene in these noble gas matrices, shows strong shifts (up to 500 cm^{-1}) depending on the choice of the gas. This all indicates that low temperature, inertness and optical transparency alone are not sufficient to explain the peculiar sharpness of the quasi-linear spectra obtained. In Chapter II the speculations and experimental evidence concerned with how the guest molecule and matrix behave have already been detailed.

It still remains an open question as to how to predict which solvents will permit observations of the Shpol'skii effect. According to Shpol'skii[24], in solvents where hydrogen bonds are possible, as in methanol or ethanol, the fluorescence spectra of aromatic hydrocarbons are highly diffuse even at low temperature. In higher alcohols such as n-hexanol, the role of the OH group, which is responsible for the hydrogen bonding, is decreased, because of the length of the hydrocarbon part of the molecule. In an n-hexanol matrix at 77 K, Shpol'skii[24] observed the quasi-linear spectrum of pyrene. No spectrum was, however, found in the literature consulted and neither has further information been disclosed concerning this matrix.

Other solvents have been found to produce quasi-linear luminescence spectra, Sponer, Kanda and Blackwell[193] have used cyclohexane to study the quasi-linear phosphorescence spectra of benzene at 77 K and at 4.2 K. Also in a cyclohexane matrix, Janić and Kawski[194] obtained a quasi-linear spectrum from isoquinoline. Pellois, Ripoche and Collorec[195,196] studied pyrene and 1,12-benzoperylene in methylcyclopentane at 88 K and 9 K. Val'dman and Sheremet'ev[197] observed a narrow line spectrum for perylene in dibenzylaminoethanol (which is crystalline at room temperature) at 20.4 K; at 77 K, however, the spectrum was diffuse in character.

Nurmukhametov[43] showed that it was possible to obtain a quasi-linear spectrum in a polymeric matrix when pyrene was studied in poly-2-methylpropene. Grebenshchikov, Kovrizhnykh and Kozlov[198] studied the phosphorescence spectra of triphenylene, hexahydrotriphenylene, coronene, diphenylene oxide and diphenylene sulphide in 2,2,4-trimethylpentane and in carbon tetrachloride at 77 K. According to Grebenshchikov and co-workers[198], in carbon tetrachloride all compounds investigated, except hexahydrotriphenylene, showed quasi-linear spectra. Coronene in 2,2,4-tri-methylpentane presented a similar spectrum, in its vibrational structure, to the spectrum in n-heptane; the spectra of other compounds in 2,2,4-trimethylpentane consist of bands against the background of which lines can be distinguished; the lines, however, are somewhat wider than in n-paraffin[198] solvents.

Experimental

A preliminary study was undertaken to investigate the use of other

solvents matrices for the production of the Shpol'skii effect. The model polycyclic aromatic compound used in the study was coronene; this hydrocarbon exhibits a relatively simple and well defined quasi-linear emission in n-hexane, n-heptane and n-octane. Coronene also exhibits both fluorescence and phosphorescence and has the advantage of being quite harmless, when pure. Also due to all of these qualities coronene can be recommended as a standard for testing instrumentation used in quasi-linear spectrometry.

The emission spectra of solutions containing 20 $\mu g\ ml^{-1}$ of coronene in 1,4-dioxan, n-pentanol, carbon tetrachloride, chloroform, bromoform, diethylether, dimethylformamide, 1,1,2,2-tetrachlorethane, 1-bromo-octane, tetrahydrofuran, furan and propylene carbonate were examined at 77 K.

Instrument

This study was started with the instrumental arrangement described in Chapter IV and continued with the instrumental arrangement described in Chapter V; most of the experiments were repeated with the latter arrangement.

Materials and Reagents.

The sources of the polycyclic aromatic hydrocarbons are the same as described earlier (see Chapter IV). The other compounds tested were of Reagent Grade and of diverse origin: the alkaloids supplied by BDH

Chemicals Ltd. Quinoline and isoquinoline (BDH) were purified using low pressure distillation. Lawsone was obtained from Fluka Laboratories. Phthiocol, lapachol and norlapachol were obtained or synthesized by us as described in our previous work[199]. Aflatoxins were kindly donated by Dr. R.D. Stubblefield, Agricultural Research Service, Illinois, U.S.A. The vitamins used were either from BDH or Merck. Nalidixic acid was purchased from Sigma London Chem. Co.

The n-paraffins solvents (BDH) were purified by percolation through activated silicagel and tetrahydrofuran (Hopkin and Williams Ltd.) was purified by treatment with KOH, distillation, further treatment with Na-wire and redistillation.

Results

Only with tetrahydrofuran (THF) was a well defined quasi-linear emission spectrum observed for coronene. Both quasi-linear fluorescence and phosphorescence were observed (Figures VIII - 1a and VIII - 2).

Table VIII - 1 summarises the observations with the other solvents studied; in some solvents other polycyclic aromatic hydrocarbons (PAH) were also tested without success. The lack of quasi-linear emission in the spectrum of coronene in carbon tetrachloride is probably due to the fact that we consider the appearance of quasi-linear emission, which is not a very precise term, where the half-band width is less than 1 nm. Probably an alternative and better way to define the appearance of the Shpol'skii effect is to compare the half-band width obtained in the matrix in question with the half-band width of the same compound in a glassy matrix such as EPA (ether: iso-pentane: ethylalcohol;

TABLE VIII - 1

Solvent	Appearance at 77 K	Compound tested	Observations
Furan	Cracked Polycrystalline	Coronene[1]	All compounds presented broad band emission
		3,4-Benzopyrene	
		3 -Methyl-cholanthrene	
1,4-Dioxan	Polycrystalline	Coronene[2]	Narrow band fluorescence emission on top of broad peak. No phosphorescence
		Pyrene	Broad band fluorescence emission
		3,4-Benzopyrene	Very broad band emission. Maximum at 409.5 nm
n-Pentanol	Cracked glass	Coronene	Broad band emission
Dimethyl-formamide	Polycrystalline	Coronene	Strong intensity, both fluorescence and phosphorescence. Broad band emission
Carbon tetrachloride	Polycrystalline	Coronene	Weak fluorescence signal. Medium intensity phosphorescence signal. Broad
Chloroform	Polycrystalline	Coronene	Strong phosphorescence signal. Weak fluorescence signal. Broad.
1-Bromo-octane	Polycrystalline	Coronene	Weak fluorescence. Strong phosphorescence. Broad band emission
		Triphenylene	Strong phosphorescence signal. Broad emission.
		3,4-Benzopyrene	Very broad band fluorescence signal.

Solvent	Appearance at 77 K	Compound tested	Observations
1,1,2,2-Tetra	Polycrystalline	Coronene	Strong phosphorescence - weak fluorescence.
chloroethane			Both broad
Propylene	Cracked	Coronene	Broad band emissions
carbonate	Polycrystalline	3,4-Benzopyrene	

Other compounds were also tested such as 1. 1,2-benzopyrene, 1,2,5,6-dibenzanthracene; 2. 1,2-dibenzanthracene, 1,2,5,6-dibenzanthracene, 3,4,8,9-dibenzopyrene, 3,4,9,10-dibenzopyrene and 3-methylcholanthrene; all produced broad band emission.

5:5:1 v/v). Figure III-2 shows for instance the effect in changing from EPA to n-paraffin matrix, using pyrene. The emission of coronene in carbon tetrachloride appears to have a half-band width much higher than 1 nm, both in our results and in the spectrum published by Butlar and Grebenschchikov[200].

With the other cyclic ethers tested (furan and 1,4-dioxan) no quasi-linear emission was observed, in contrast with THF. In furan, which produces a cracked polycrystalline matrix, the compounds investigated presented a broad band emission; coronene in 1,4-dioxan displayed a narrow band on top of a broad peak. As expected all halogenated solvents quenched the fluorescence to some extent (due to heavy atom effect) and enhanced the phosphorescence emission.

Our finding that it is possible to obtain a quasi-linear emission spectrum with coronene in tetrahydrofuran led us to search for other compounds which showed quasi-linear emission in such a matrix, and how this matrix compares with the usual Shpol'skii matrices(the n-paraffin solvents).

Polycyclic aromatic hydrocarbons (PAH)

Firstly thirty-one polycyclic aromatic hydrocarbons were studied in a tetrahydrofuran matrix. Quasi-linear emission was observed for 1,2-benzopyrene, 1,2,5,6-dibenzanthracene, 4,9-di-t-butylpyrene, and ovalene (Figures VIII - 1c, 3a and 4b).

Spectral emission band widths that were greater than those

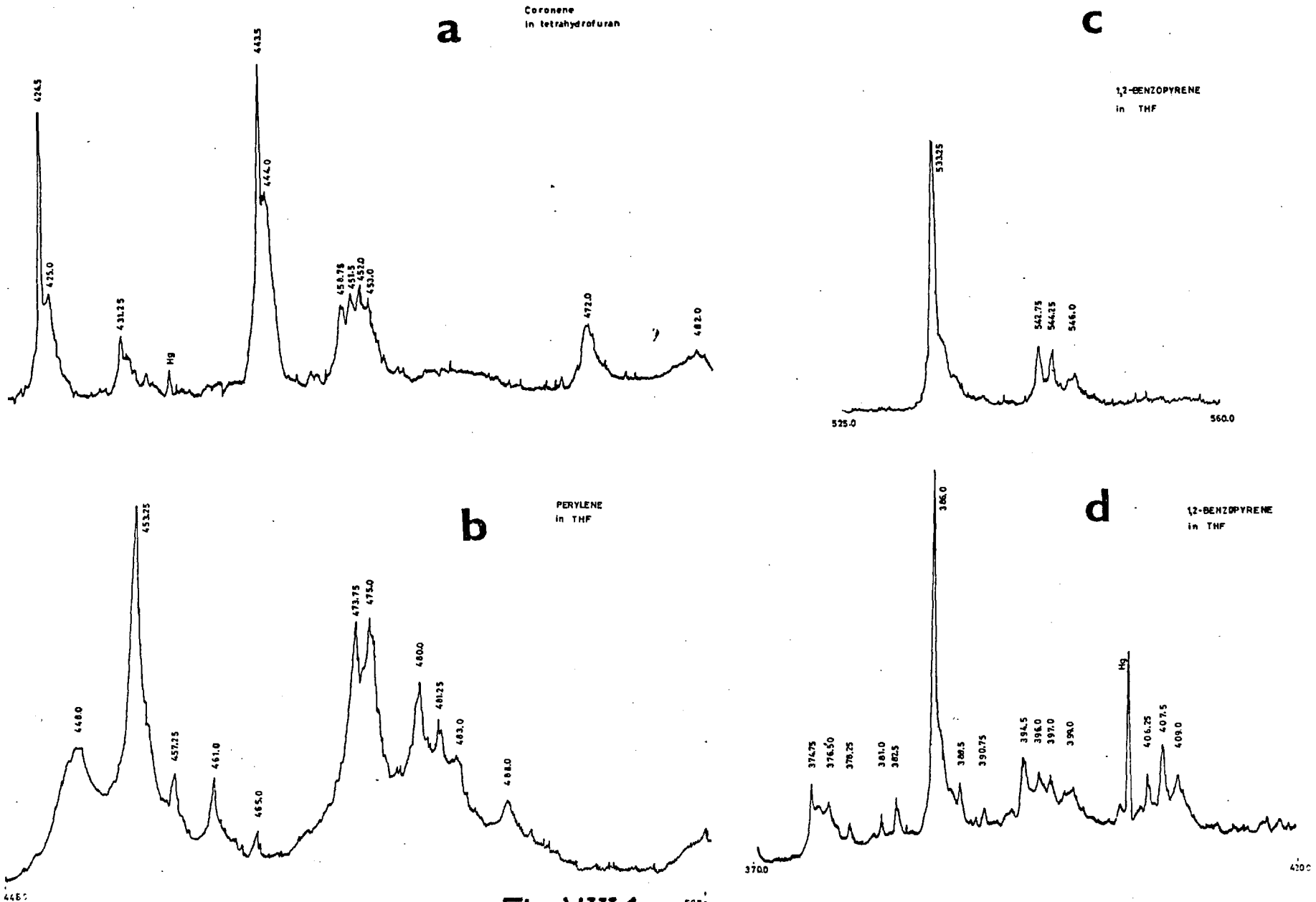

a
Coronene
in tetrahydrofuran
424.5
425.0
431.25
Hg
443.5
444.0
456.75
457.5
459.0
460.0
472.0
482.0
b
PERYLENE
in THF
448.0
453.25
457.25
461.0
465.0
473.75
475.0
480.0
481.25
483.0
488.0
c
1,2-BENZOPYRENE
in THF
533.25
542.75
544.25
546.0
525.0
560.0
d
1,2-BENZOPYRENE
in THF
386.0
374.75
376.50
378.25
381.0
382.5
388.5
390.75
394.5
396.0
397.0
398.0
Hg
406.25
407.5
409.0
370.0
420.0

Fig.VIII-1

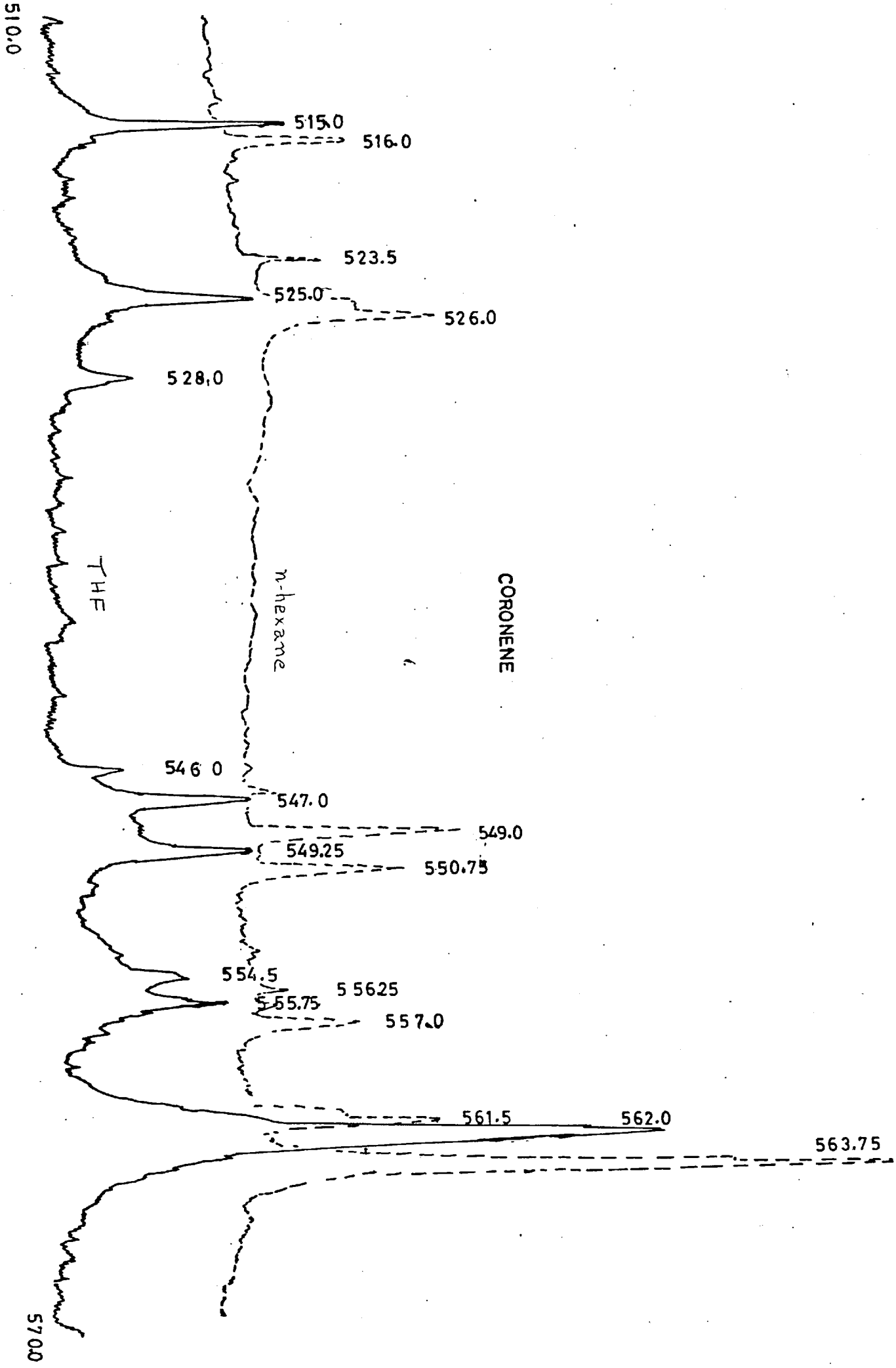

510.0
515.0
516.0
523.5
525.0
526.0
528.0
THF
n-hexane
CORONENE
546 0
547.0
549.0
549.25
550.75
554.5
5 5625
55.75
557.0
561.5
562.0
563.75
570.0

Fig. VIII-2

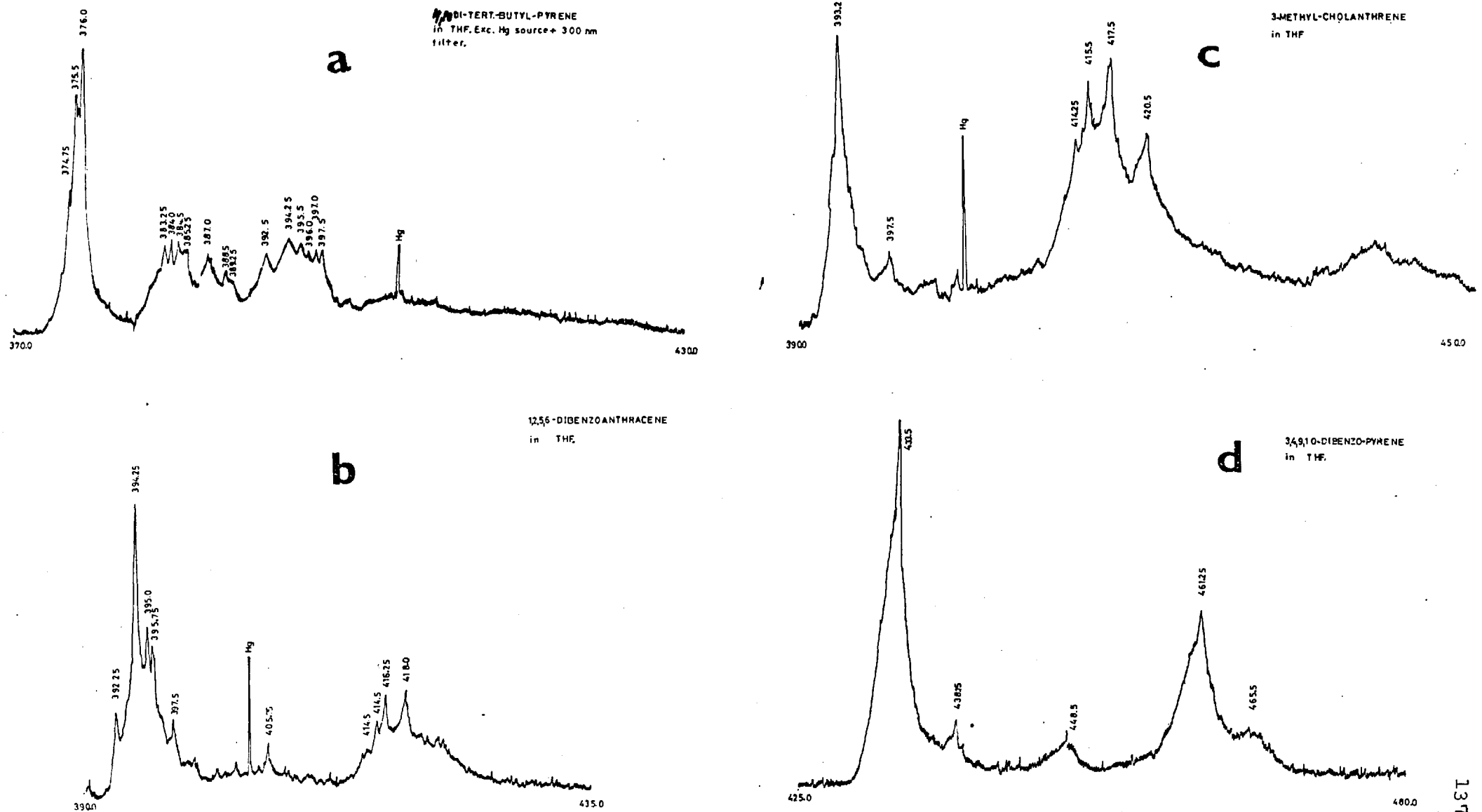

a
DI-TERT.-BUTYL-PYRENE
in THF. Exc. Hg source + 300 nm
filter.
374.75
375.5
376.0
381.25
382.0
384.5
385.25
387.0
388.5
392.5
394.25
395.5
396.0
397.0
397.5
Hg
370.0
430.0
b
12,5,6-DIBENZOANTHRACENE
in THF.
392.25
394.25
395.0
395.75
397.5
Hg
405.75
414.5
414.5
416.25
418.0
390.0
435.0
c
3-METHYL-CHOLANTHRENE
in THF
393.25
397.5
Hg
414.25
415.5
417.5
420.5
390.0
450.0
d
3,4,9,10-DIBENZO-PYRENE
in THF.
428.5
438.25
448.5
461.25
465.5
425.0
480.0

Fig. VIII-3

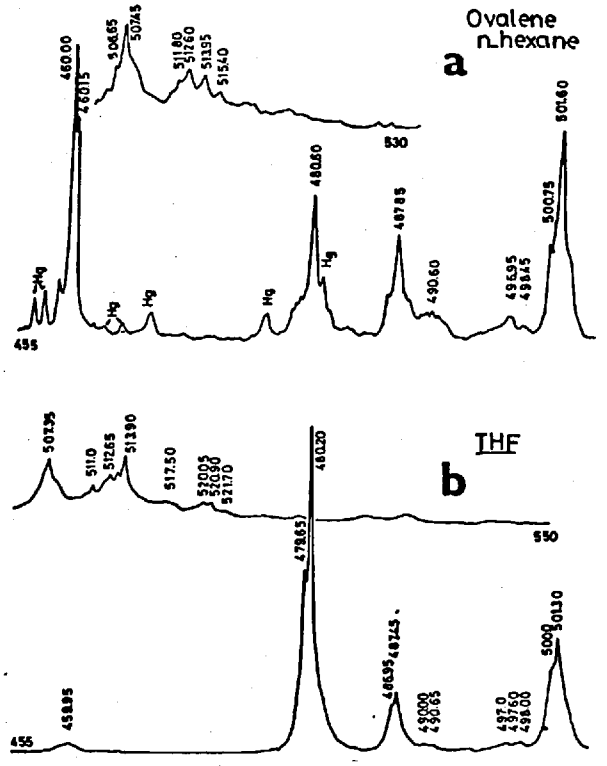


Fig. VIII-4

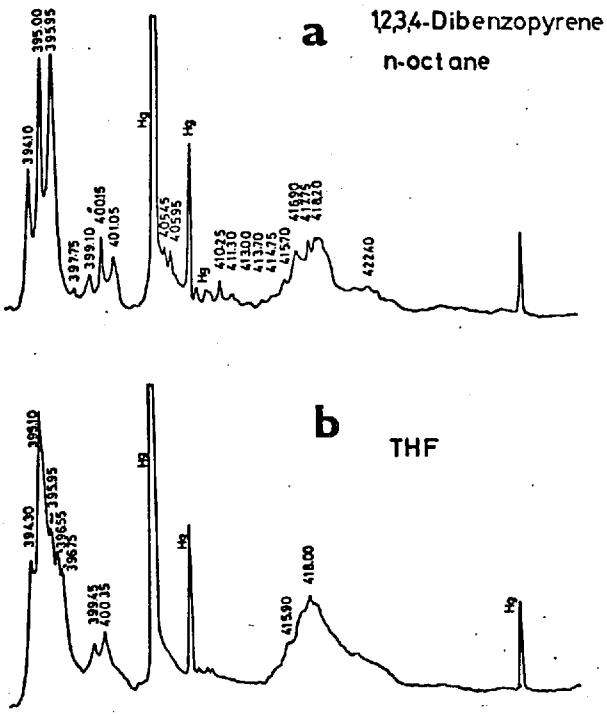


Fig. VIII-5

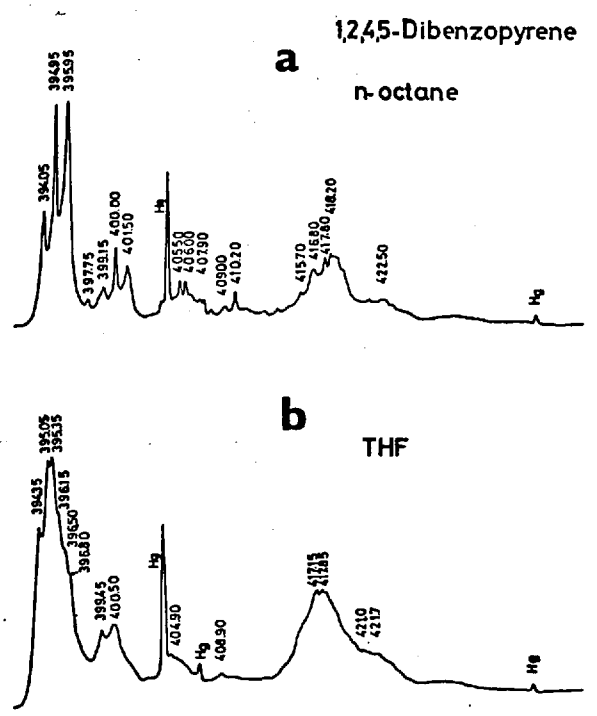


Fig. VIII-6

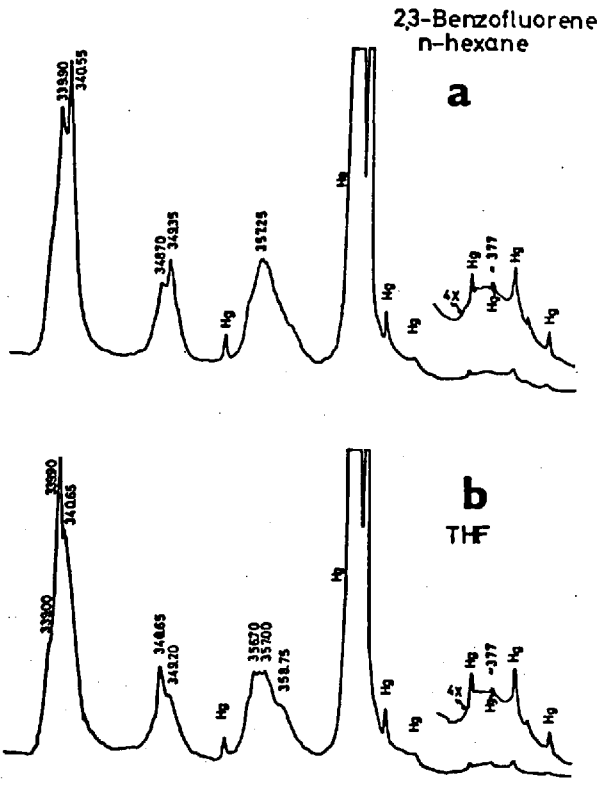


Fig. VIII-7

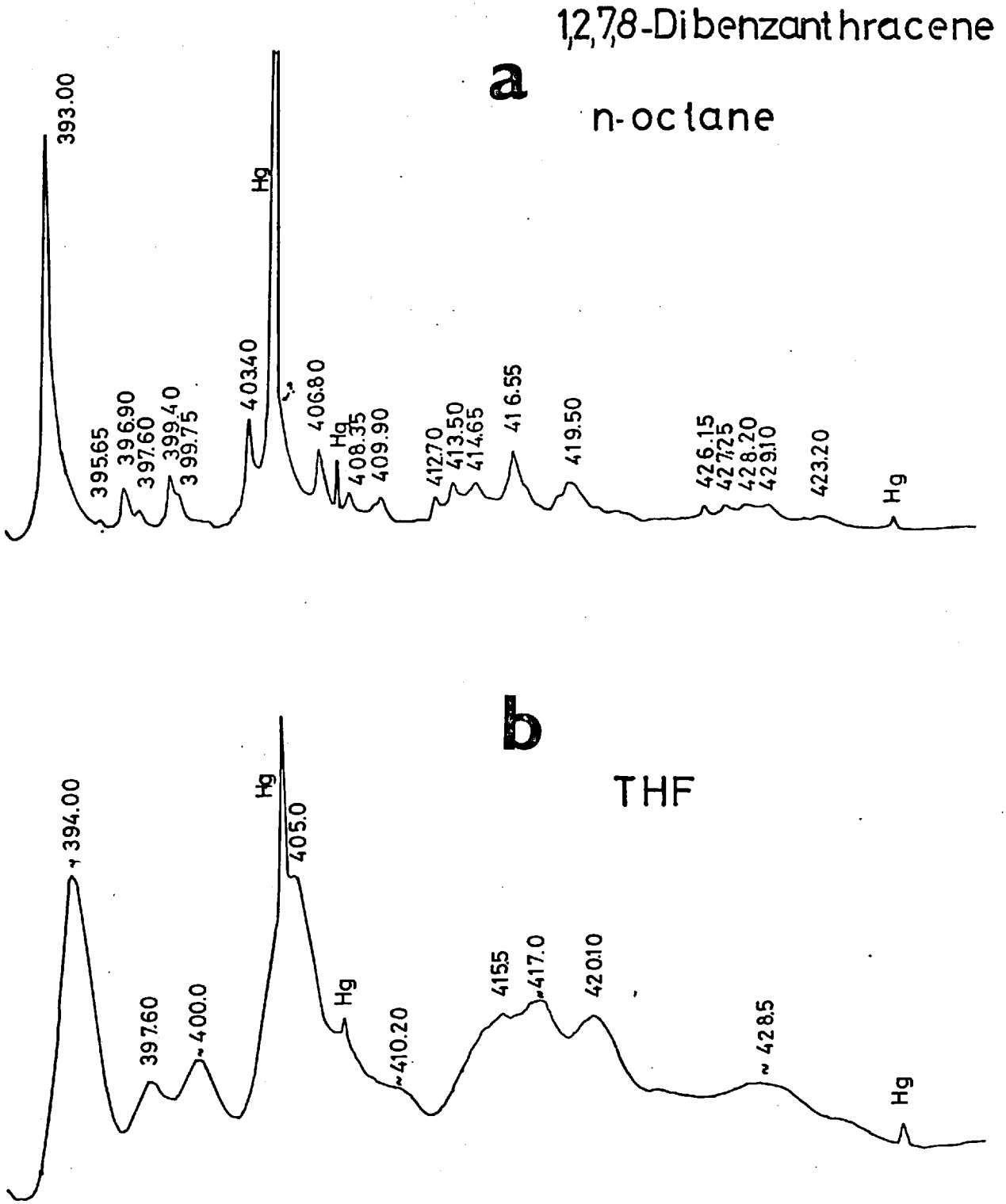

1,2,7,8-Dibenzanthracene
a
n-octane
393.00
Hg
395.65
396.90
397.60
399.40
399.75
403.40
406.80
Hg
408.35
409.90
412.70
413.50
414.65
416.55
419.50
426.15
427.25
428.20
429.10
423.20
Hg
b
THF
394.00
Hg
405.0
397.60
400.0
Hg
410.20
415.5
417.0
420.10
428.5
Hg

Fig.VIII-8

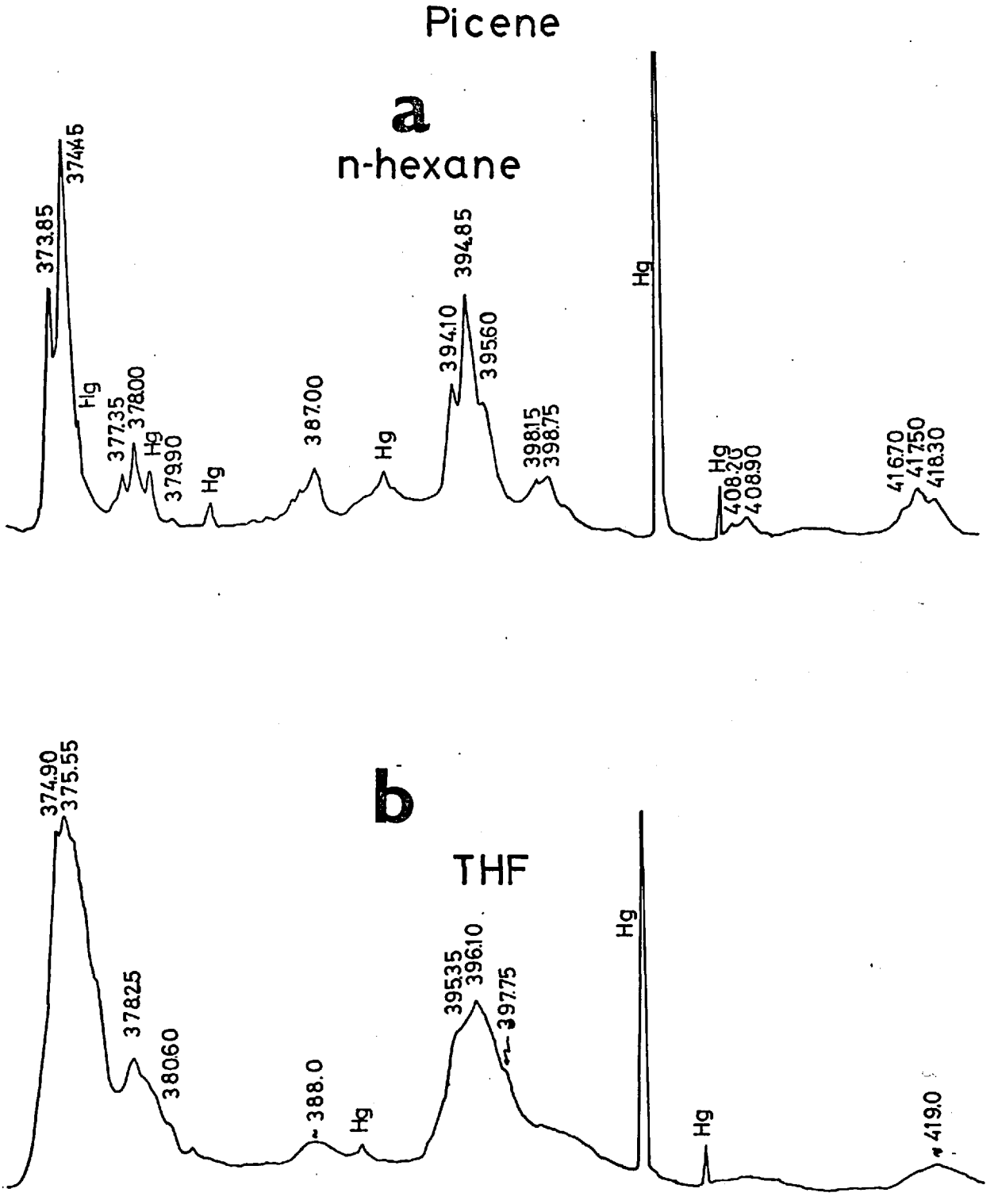

Picene
a
n-hexane
373.85
374.45
Hg
377.35
378.00
Hg
379.90
Hg
387.00
Hg
394.10
394.85
395.60
398.15
398.75
Hg
Hg
408.20
408.90
416.70
417.50
418.30
b
THF
374.90
375.55
378.25
380.60
388.0
Hg
395.35
396.10
397.75
Hg
Hg
419.0

Fig.VIII-9

expected for the Shpol'skii effect were observed for perylene (Fig. VIII - 1b) 3-methylcholanthrene, 3,4,9,10-dibenzopyrene (Fig. VIII - 3c,d), 1,2,3,4-dibenzopyrene (Fig. VIII - 5b), 1,2,4,5-dibenzopyrene (Fig. VIII - 6b), 1,12-benzoperylene, 2,3-benzofluorene (Fig. VIII - 7b) and 1,2,3,4-dibenzanthracene.

Sometimes the band width seems to be the result of an overlap of multiplets, e.g. in the 1,2,3,4-dibenzopyrene and 1,2,4,5-dibenzopyrene spectra (Figures 5b and 6b); in such cases we can consider that the Shpol'skii effect is also present.

Examples of broad band emission in THF can be seen in the spectrum of 1,2,7,8-dibenzanthracene (Fig. VIII - 8b) and in the spectrum of picene (Fig. VIII - 9b) which can be compared with the quasi-linear spectrum of the same compounds in n-octane (1,2,7,8-dibenzanthracene - Fig. VIII - 8a) and in n-hexane (picene - Fig. VIII - 9a).

Table VIII - 2 shows the principal emission characteristics at 77 K of some of the compounds tested in tetrahydrofuran.

Compounds other than Polynuclear Aromatic Hydrocarbons

Some simple heterocyclic compounds were tested in tetrahydrofuran. Quasi-linear phosphorescence was observed for diphenylene oxide (dibenzofuran Fig. VIII - 10b), quinoline and isoquinoline (Fig. VIII - 11) although most of these compounds also gave broad band or diffuse fluorescence emission.

Carbazole produced very broad band fluorescence and phosphorescence spectra, although in n-heptane this compound produces a quasi-linear

TABLE VIII - 2 Emission at 77 K of some PAH compounds in tetrahydrofuran

COMPOUND	Wavelengths of Principal Emission Maxima
Coronene	424.5(s), 425.0(m), 431.25(w), 443.5(vs), 444.0(s), 450.75(m), 451.5(m), 452.0(m), 453.0(m), 472.0(m), 482.0(w), P: 515.0(m) 525.0(m), 528.0(w), 547.0(m), 549.25(m), 554.5(m), 555.75(m), 562.0(vs).
Perylene	448.0(m), 453.25(vs), 457.25(m), 461.0(m), 465.0(w), 473.75(s), 475.0(s), 480.0(m), 481.25(m), 483.0(m), 488.0(w).
1,2-Benzopyrene	374.75(m), 376.50(m), 378.25(w), 381.0(w), 382.5(m), 386.0(vs), 388.5(m), 390.75(w), 394.5(m), 396.0(m), 397.0(m), 399.0(m), 406.25(m), 407.5(m), 409(m). P: 533.25(s), 542.75(m), 544.25(m), 546.0(w)
3,4,9,10-Dibenzopyrene	433.5(vs), 438.75(w), 448.5(w), 461.25(m), 465.5(d.w)
3-Methyl-cholanthrene	393.25(vs), 397.5(w), 414.25(m), 415.5(m), 417.5(m), 4205(m)
1,2,5,6-Dibenzanthracene	392.25(m), 394.25(vs), 395.0(m(, 397.5(w) 405.75(w), 414.5(vw), 415.5(w), 416.25(m), 418.0(m).
4,9-Di-t-butylpyrene	374.75(m), 375.5(s), 376.0(vs), 383.25(m), 384.0(ms), 384.5(m), 385.25(m), 387.0(m(, 388.5(m), 389.25(w), 392.5(m), 394.25(m), 395.5(m), 396.0(m), 397.0(m), 397.5(m).

Ovalene	459.95(vw), 479.65(m), 480.20(vs), 486.95(m), 480.45(m), 490.00(vw), 490.65(vw), 497.0(vw), 497.60(vw), 500.0(m), 501.30(m).
1,2,3,4-Dibenzopyrene	394.30(s), 395.10(vs), 395.95(s), 396.75(m), 399.45(m), 400.35(m), 415.90(m), 418.0(m).
1,2,4,5-Dibenzopyrene	394.35(s), 395.05(vs), 395.35(vs), 396.15(s), 396.50(m), 396.80(m), 399.45(m), 400.5(m), 404.90(w), 408.90(vw), 417.15(m), 417.85(m), 421.0(w), 421.7(w).
2,3-Benzofluorene	339.00(m,Sh), 339.90(vs), 340.65(s), 348.65(m), 349.20(m), 356.70(m), 357.00(m), 358.75(m).
Diphenylene oxide	P: 405.40(vs), 408.75(w), 416.05(vw), 417.80(w), 419.60(w), 422.65(w), 424.0(w), 426.50(w), 427.80(w), 434.10(s), 438.0(w), 480.0(w).
Quinoline	P: 453.90(vs), 465.0(m), 467.25(w), 471.35(w), 476.25(w), 478.50(vw), 484.25(m), 488.80(m), 496.75(w).
Isoquinoline	P: 453.90(w), 468.15(s), 477.10(s), 479.50(m), 486.15(w), 487.70(vs), 490.25(s), 499.80(w), 500.75(w).

w - weak; vw - very weak; s - strong, vs - very strong, m - medium, sh - shoulder, P - phosphorescence emission, d - diffuse.

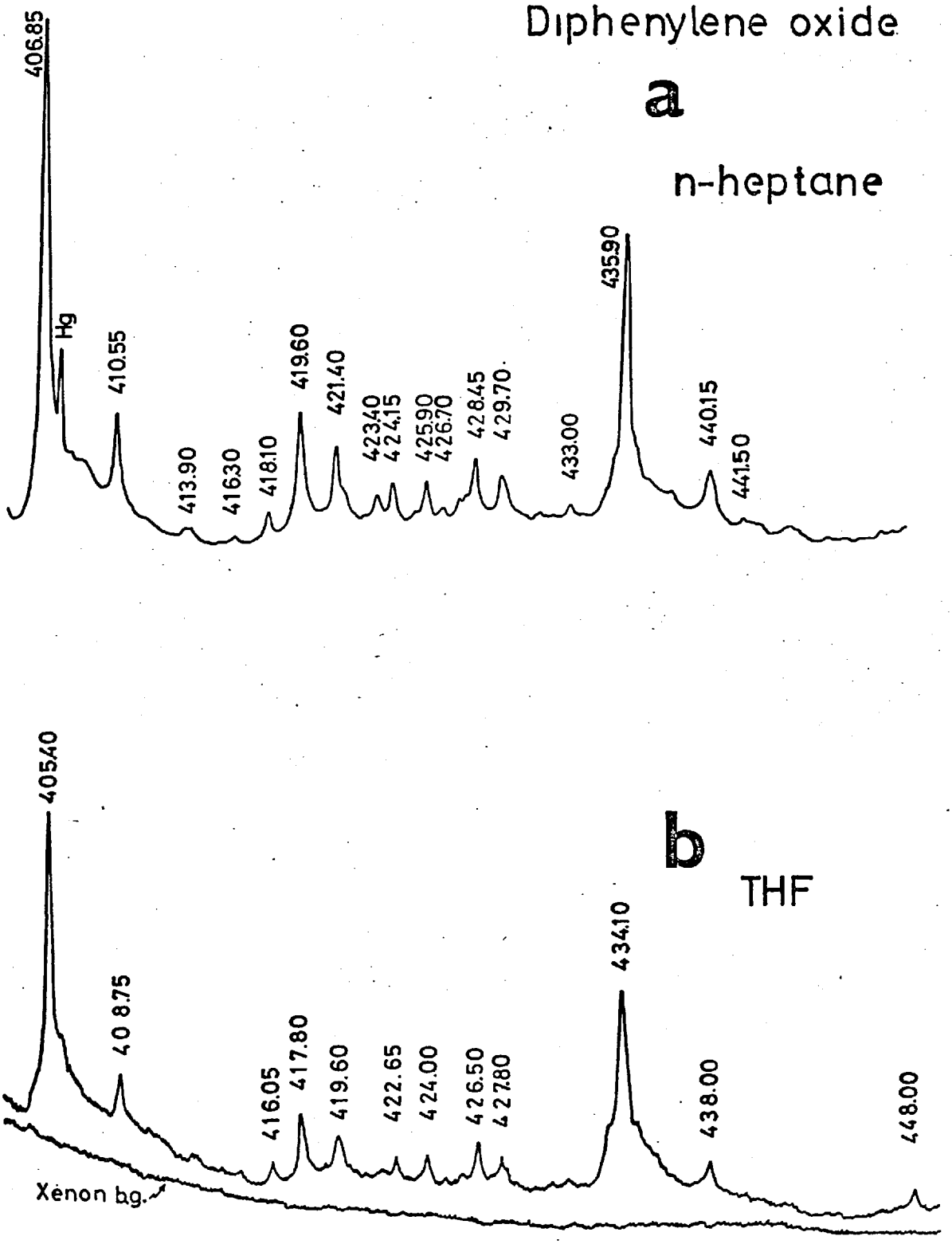

Diphenylene oxide
a
n-heptane
406.85
Hg
410.55
413.90
416.30
418.10
419.60
421.40
423.40
424.15
425.90
426.70
428.45
429.70
433.00
435.90
440.15
441.50
b
THF
405.40
408.75
416.05
417.80
419.60
422.65
424.00
426.50
427.80
434.10
438.00
448.00
Xenon bg.

Fig. VIII-10

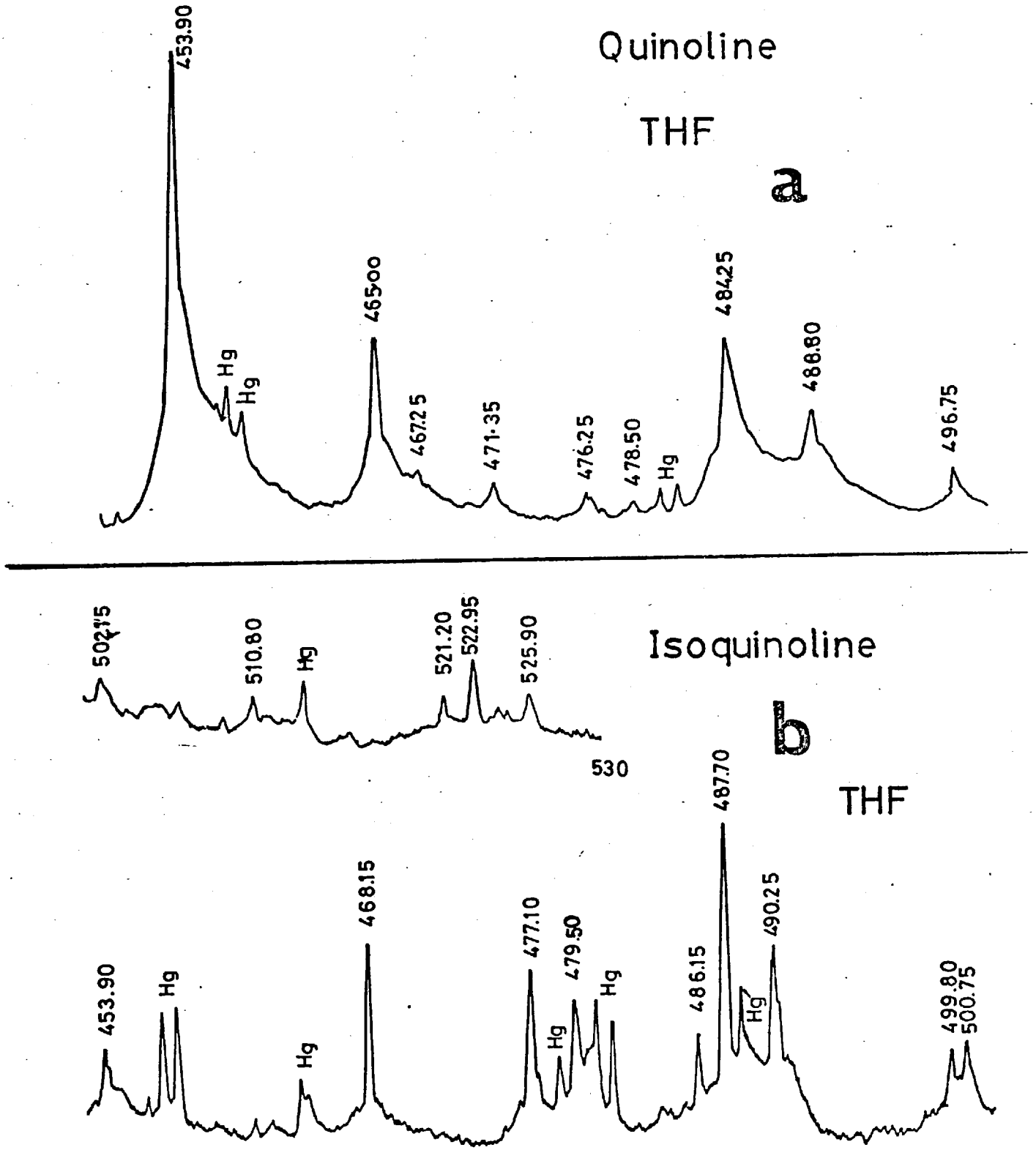
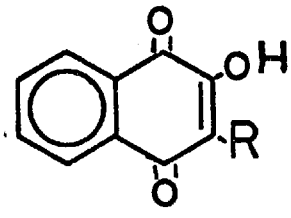

453.90
Hg
Hg
465.00
467.25
471.35
476.25
478.50
Hg
484.25
488.80
496.75
Quinoline
THF
a
502.15
510.80
Hg
521.20
522.95
525.90
530
Isoquinoline
b
THF
453.90
Hg
Hg
468.15
477.10
Hg
479.50
Hg
486.15
487.70
Hg
490.25
499.80
500.75

Fig. VIII-11

emission spectrum. Neither indole nor phenazine produced a luminescence signal. 2-Amino-chrysene showed a strong diffuse fluorescence signal between 400-440 nm with a maximum at 429.0 nm. 2,6-Di-hydroxy-naphthalene presented also a very diffuse signal (between 365 and 430 nm) with maxima at 405.0 and 417.0 nm.

Naphthoquinones

1,4-Naphthoquinone and four 3-alkyl - 2-hydroxy - 1,4-naphthoquinones (lawsone, phthiocol, lapachol and norlapachol) were tested in THF, but the luminescence signal either was very weak or was not

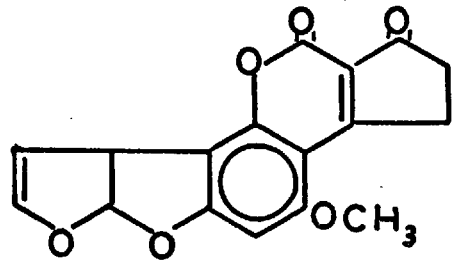


R=-H (lawsone)
R=-CH_3 (phthiocol)
R=-CH_2-CH=$C(CH_3)_2$ (lapachol)
R=-CH=$C(CH_3)_2$ (norlapachol)

detected.

Aflatoxins

Aflatoxins, which are carcinogenic mold metabolites, are sometimes present in agricultural food products and usually are detected by fluorescence spectrometry. Aflatoxins B_1, B_2, G_1 and G_2 were tested in THF. Although strong luminescence was present only diffuse or very diffuse emissions were observed.



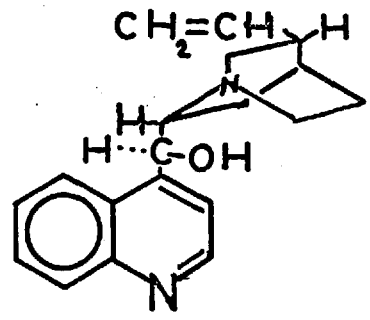
aflatoxin B_1

Aflatoxins B_1 and B_2 show emission between 400 and 500 nm; B_1 showed a maximum at 464.0 nm and B_2 showed maxima at 464.0 and 475.0 nm. The aflatoxin G_1 showed maxima at 415.0 and 485.0 nm; G_2 presented a maximum at 413.6 nm.

Alkaloids

A large number of alkaloids were tested in THF. Either no luminescence or very weak luminescence was present (e.g. papaverine, theobromine, strychnine, nicotine, morphine, codeine, brucine, atropine and digitaline) or the luminescence signal was very diffuse as with

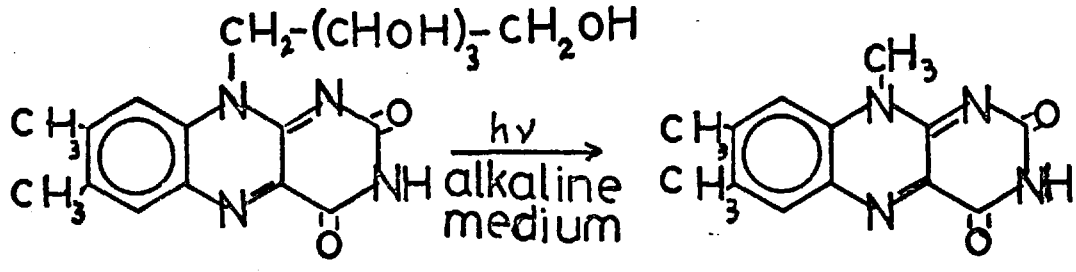
berberine, narcotine, cocaine and aconitine. Cinchonidine, cinchonine and quinidine showed more narrow, but still very broad band emission, which suggested the study with quinoline (which showed quasi-linear emission) which is the aromatic structure present in these alkaloids.



cinchonidine

Vitamins

Vitamins A_1, B_2 (riboflavin), B_{12}; K_1 and K_5 were tested but none produced quasi-linear emission in THF. Riboflavin when illuminated in an alkaline solution, splits off four atoms of the lateral chain and is converted to lumiflavin[201]. We obtained lumiflavin using this procedure and examined its behaviour in THF at 77 K; it displayed a diffuse band spectrum.



riboflavin lumiflavin

Antibiotics

Only one antibiotic (nalidixic acid) was tested in THF. Blue phosphorescence was observed between 390 and 475 nm but this was very diffuse and exhibited a maximum at 400 nm.

Discussion

This large list of compounds tested, of which most failed to produce narrow or quasi-linear spectra, raises the question related to the Shpol'skii effect: what are the characteristics of compounds which favour the appearance of the effect?

General and reliable rules establishing a relationship between the structure of the molecules and the possibility of the occurrence of quasi-linear emission are not yet known[34]. Sometimes the first attempts made with one compound fail only to be successful later; e.g. chlorophyll failed to produce quasi-linear emission at the first attempt but later at 4 K, and using a concentration of 1×10^{-6} M it was possible to obtain both quasi-linear absorption and fluorescence[34]. Precipitation due to insolubility during the freezing process has, also, in the first attempts prevented the appearance of signals; phthalimide and its N-substituted derivatives suffered such problems in a study made by Nurmukhametov[43].

Although no general rules can be established it has been observed that molecules with a rigid planar structure are more likely to produce quasi-linear emission or absorption spectra. Also the lack of intramolecular or intermolecular interaction is a necessary condition;

compounds which are able to form hydrogen bonds show broad or diffuse band spectra. Shigorin, Shcheglova and Nurmukhametov[202] observed, during the study of the effect of substituents in the phosphorescence spectrum of anthraquinone, that the introduction of an OH group in the α-position resulted in a diffuse spectrum. This was attributed to intramolecular hydrogen bonding with another substituent such as methyl-,ethyl- or methoxy. Little change was observed in the spectrum although the presence of halogen substituents in the α-position also caused some diffuse character. When the OH group was introduced in the β-position a diffuse spectrum was again observed; this was attributed, to inter-molecular hydrogen bonding and consequent association of the molecules, even at the low concentrations used[202].

Fenina[203], studying the fluorescence spectra of 1,2-benzanthracene and its monomethyl derivatives, observed that when the methyl group was substituted in the 9 and 1' positions which are sterically hindered, it produced a marked decrease in the narrowness of the spectrum.

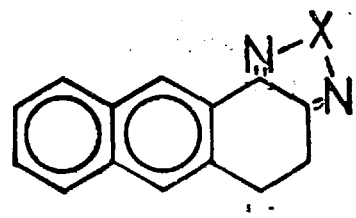
Utkina[43] studying anthracence derivatives observed that the spectrum became less narrow the greater the steric hindrance of the molecule. Utkina and Fenina[204] studying the fluorescence and absorption spectra of monomethyl derivatives of 3,4-benzophenanthrene in n-paraffin matrices (from n-hexane to n-nonane) observed that 1methyl-3,4-benzophenanthrene presented a diffuse spectrum in both fluorescence and absorption. This diffuse spectrum was explained as caused by an increase in the steric stress and lost of planarity.

Nurmukhametov, Mileshina and Malkes[45] observed that the poor resolution of some diaryldivinylbenzenes is due to the non-planarity of the molecules. The same workers made attempts to obtain fine structure

with aromatic alcohols and ethers, such as phenol, naphthols, anisole, etc., in n-paraffin solution; these failed.

According to Nurmukhametov[43], when in an amino substituted molecule the amino-group is not included in the rigid framework and is capable of rotation in relation to the C-N axis (as in aniline, diphenyl-aniline, triphenylamine), it is not possible to observe greater resolution in the spectrum in n-hydrocarbon matrices in comparison with glassy matrices. When the amino-group is rigidly fixed in the molecule, however, quasi-linear spectra can be observed, e.g. carbazole and acridan (9,10-dihydroacridine) in some n-paraffin matrices.

Nevertheless, a very large number of compounds have been shown to produce quasi-linear spectra-polyphenyls, diphenylpolyenes[205], arylacetylenes[43], benzoxazoles[206] and naphthoxazoles[210], phthalimides and naphthalimides[43], thioindigoids and indigoids[207], porphyrins and phthalocyanines[85,208] xanthones[43] and aromatic compounds with either sulfur or selenium in the molecule[209] such as



X=S or Se

although, due to the problems already described not necessarily all members of one family produce quasi-linear emission.

The restrictions imposed for the appearance of the Shpol'skii effect from the point of view of the solute molecule probably explain

the lack of narrow or quasi-linear emission in all of the polar compounds studied in THF. The lack of compatibility between the solute molecule and the solvent molecule, plays an important role, when hydrogen bonding is not present as, for instance, in the case of the polycyclic aromatic hydrocarbons studied in THF which did not produce quasi-linear emission. Also the possibility of interaction between the compounds which are polar and the THF matrix cannot be ruled out, leading to a decrease in the narrowness of the spectra.

Tetrahydrofuran and the n-paraffin solvents

Table VIII-3 and Table VIII-4 show the results for the principal luminescence emission maxima obtained for some of the compounds studied, in both THF and selected n-paraffin solvents. In a comparison of the fluorescence spectra of the compounds which produced narrow band or quasi-linear emission in THF with the spectra obtained for these compounds in some n-paraffin solvents we observed small shifts in the wavelengths of maximum emission (exception made in the case of isoquinoline when the comparison was made using the lowest wavelength emission and which was not a maximum) were either to the red or to the blue; in a few cases no shift was observed.

The relative intensities of the emission maxima in the quasi-linear or narrow band spectra observed in THF are usually different to those observed in the n-paraffin solvents. As a general case, however, when a particular PAH compound shows quasi-linear, narrow band fluorescence or phosphorescence emission in THF it is usually found to exhibit this type of behaviour in n-hexane or n-heptane. Examples of these similarities

TABLE VIII-3

COMPOUND	Fluorescence Emission in THF			Fluorescence Emission in n-paraffin			
	H.B.W.[a]	λ, nm	ν, cm^{-1}	λ, nm	ν, cm^{-1}	N-Cn[b]	$\Delta\nu \pm 5$[c]
2,3,-Benzofluorene	87	339.90	29,420	340.55	29,364	6	+56
Carbazole	382	343.50	29,112	340.75	29,347	7	-235[1]
Phenanthrene	167	346.00	28,902	345.50	28,943	6	-41
Triphenylene	65	352.45	28,373	352.25	28,390	8	-17
"	65	352.45	28,373	352.10	28,401	6	-28
Chrysene	230	~361.50	27,662	360.50	27,739	6	-77
Pyrene	51	371.25	26,936	371.75	26,900	8	+36
3,4-Benzophenanthrene	214	~374.20	26,724	373.758	26,755	8	-32[2]
Picene	71	375.55	26,627	374.45	26,706	6	-79
3-Methylpyrene	142	375.25	26,649	375.00	26,667	8	-18
1,2,3,4-Dibenzanthracene	106	375.50	26,631	375.05	26,663	8	-32
4,9-di-t-Butylpyrene	29	376.00	26,596	375.00	26,667	8	-71
"	"	"	"	375.15	26,656	6	-60[3]
1,2-Benzanthracene	270	~385.00	25,974	383.75	26,059	8	-85
1,2-Benzopyrene	27	386.70	25,859	388.25	25,759	8	+102
"	"	"	"	387.55	25,803	6	+56
3,5,8,10 Tetraisopropylpyrene	206	~382.25	26,161	379.25	26,368	8	-207

COMPOUND	Fluorescence Emission in THF			Fluorescence Emission in n-paraffin			
	H.B.W.[a]	λ, nm	ν, cm^{-1}	λ, nm	ν, cm^{-1}	N-Cn[b]	Δν±5[c]
Anthracene	~800	~385.00	25,974	377.00	26,525	6	-551
3-Methylcholanthrene	97	393.75	25,397	392.55	25,474	8	-77
"	"	"	"	392.55	25,474	6	-77[3]
1,2,5,6-Dibenzanthracene	26	394.25	25,365	394.25	25,365	6	0.0
1,2,7,8-Dibenzanthracene	141	~394.00	25,381	393,00	25,445	8	-64
1,2,3,4-Dibenzopyrene	70	395.10	25,310	395.95	25,256	8	+54
1,2,4,5-Dibenzopyrene	141	395.35	25,294	395.95	25,256	8	+38
7,12-Dimethyl-1,2-benzanthracene	622	~401.00	24,938	397.75	25,141	6	-203
3,4-Benzopyrene	220	~404.60	24,716	403.00	24,814	8	-98
9,10-Dimethylanthracene	~604	~407.00	24,570	399.75	25,016	6	-446
1,12-Benzoperylene	110	405.40	24,667	406.30	24,612	8	+55
"	"	"	"	406.25	24,615	7	+52
"	"	"	"	406.00	24,630	6	+37
"	"	"	"	404.90	24,697	5	-30

COMPOUND	Fluorescence Emission in THF			Fluorescence Emission in n-paraffin			
	H.B.W.[a]	λ, nm	ν, cm^{-1}	λ, nm	ν, cm^{-1}	N-Cn[b]	$\Delta\nu \pm 5$[c]
3,4-Benzofluoranthene	539	~408.00	24,509	403.25	24,798	8	-290
Coronene	12	424.85	23,538	426.00	23,474	6	+64
3,4,9,10-Dibenzopyrene	80	433.50	23,068	431.50	23,175	8	-107
1,2-Benzonaphtho-[2'.3': 4.5] pyrene	200	~448.25	22,309	444.75	22,484	8	-175
Perylene	49	453.25	22,063	451.00	22,173	8	-110
3,4,8,9-Dibenzopyrene	428	~459.25	21,775	449.25	22,259	8	-484
Ovalene	19	480.20	20,824	480.60	20,807	6	+17
2,3-O Phenylenepyrene (Indeno (1,2,3-cd) pyrene	~634	~470.00	21,276	462.50	21,622	8	-346

(a) Half band width in cm^{-1}, (b) In the n-paraffin solvents the numbers refer to n-pentane (5) n-hexane (6) n-heptane (7) n-octane (8). (c) $\Delta\nu = \nu_{THF} - \nu_{N\text{-}C_n}$

1. Broad emission in n-heptane. 2. 70 cm^{-1} HBW in n-octane plus diffuse emission.
3. Sharper emission in n-hexane than n-octane.

TABLE VIII - 4

COMPOUND	Phosphorescence Emission in THF			Phosphorescence Emission in n-paraffin			
	H.B.W.[a]	λ, nm	ν, cm^{-1}	λ, nm	ν, cm^{-1}	N-Cn[b]	Δν[c]
Carbazole	242	406.00	24,630	408.20	24,498	7	+132[1]
Diphenylene oxide	24	405.40	24,666	406.85	24,579	7	+87
Triphenylene	71	460.95	21,694	462.25	21,633	8	+61
"	"	"	"	461.70	21,659	6	+35
Quinoline	24	453.90	22,031	459.10	21,782	6	+249
"	"	"	"	456.00	21,930	7	+101
Isoquinoline	16	453.90	22,031	459.67[2]	21,755	cC_6[2]	+276
Coronene	15	515.00	19,417	516.00	19,380	6	+37
1,2 -Benzopyrene	30	533.30	18,751	537.25	18,613	8	+138
"	"	"	"	536.65	18,634	6	+117

1. Quasi-linear emission in n-heptane cC_6 in cyclohexane
2. From Ref. 16. Other superscripts as Table VIII-3.

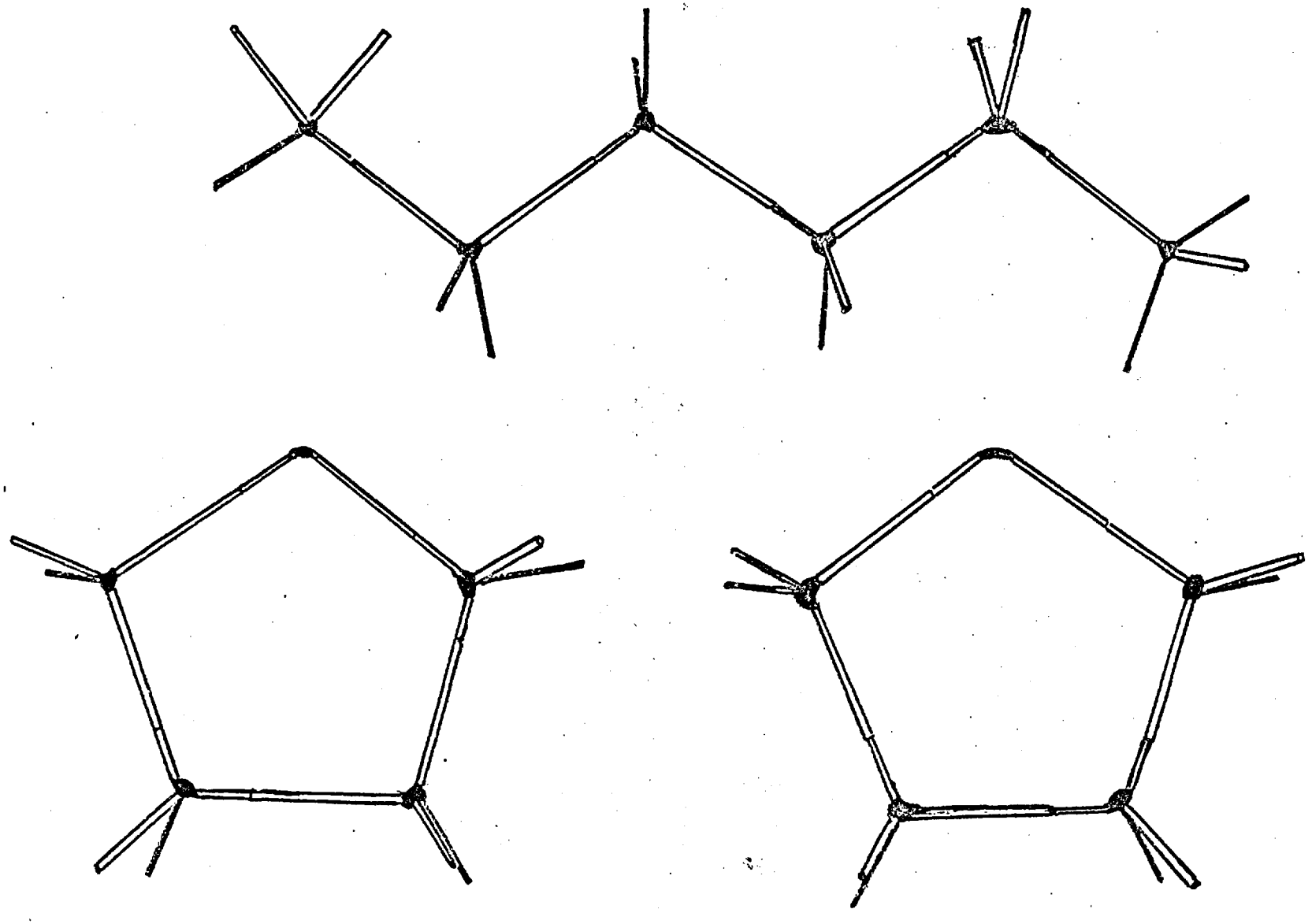


Fig. VIII-12

occur in the fluorescence emission spectra of 2,3-benzofluorene, 4,9-di-tert-butyl-pyrene, 1,2-benzopyrene, 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, coronene and ovalene. The phosphorescence emission spectra of diphenylene oxide, triphenylene and quinoline also follow this type of behaviour. Figure VIII-12 shows Dreidig^{m} models of a molecule of n-hexane (a) and two molecules of tetrahydrofuran (b) although the arrangement of the THF molecules in the Figure is not a representation of the actual crystallographic arrangement, which is not known at present.

The effect of changing the polarity from a n-paraffin solvent ($\epsilon = 2.0$) to the more polar THF ($\epsilon = 7.4$) seems to be negligible when narrow band or quasi-linear emission is present. At room temperature Veljkovic[211], studying anthracence, observed that the fluorescence shifts to the red were dependent on the dielectric constant of the solvent. In our case the principal effects observed can probably be accounted for by the low operating temperature and the degree of acceptance of the solute molecules by the frozen matrix.

Blue shifts in the emission maxima of compounds in glassy matrices have been related to the fact that solvent reorientation following excitation is more difficult in a rigid medium than in solution; this has been explained as due to the increase in the viscosity of the matrix[212]. Bhaumik and Hardwick[213] observed a blue shift with aromatic compounds embedded in plastic matrices, at ambient temperature, in relation to the emission in solution. We have observed that the fluorescence maxima of pyrene, 1,2,5,6-dibenzanthracene, 1,2-benzopyrene, 3,4-benzopyrene and coronene observed in EPA matrix are also displaced to the blue in relation to the quasi-linear emission in n-paraffin

solvents, although the emission observed in EPA was broad.

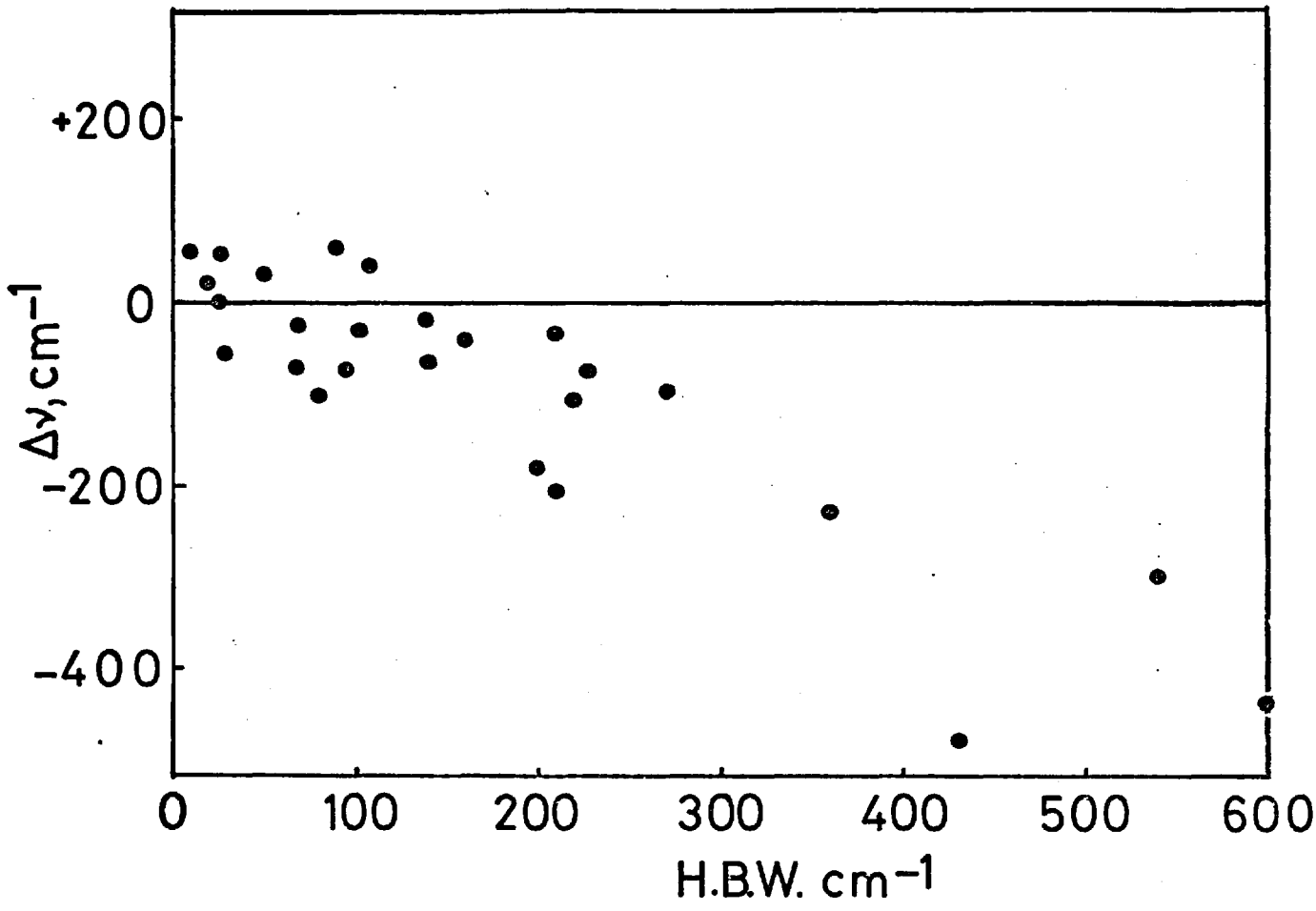


Fig. VIII - 13

Fig. VIII - 13 shows the distribution of data from Table VIII - 3) in the plot of the shift (in cm^{-1}) versus the half band width (in cm^{-1}). Although, the points are largely scattered, it is possible to see a tendency to small shifts where the half-band width (hbw) is smaller. With hbw ≤ 100 cm^{-1} the first positive shifts start to appear showing where less solute - matrix or solute-solute interactions occur.

Large red shifts compared with the n-paraffin emission and diffuse band luminescence were observed in THF with many compounds studied

e.g. chrysene, 3,5,8,10-tetra-isopropylpyrene, anthracene, 7,12-dimethyl-1,2-benzanthracene, 3,4-benzopyrene, etc; similar behaviour has been observed in n-paraffin solvents when the molecules are either deformed due to interaction with the crystal lattice or when aggregates are present[214]. The only probable effect of polarity was observed in the case of coronene where the lowest electronic transition was enhanced; similar behaviour was observed by Nakajina[215] when coronene and triphenylene emission were studied at room temperature in solvents of different polarities.

In the case of phosphorescence in the THF matrix the shifts observed relative to the n-paraffin matrices are all to the blue; as isoquinoline did not show any detectable phosphorescence in the n-paraffins tested the comparison was made vs. the published data obtained by Janić and Kawski[194] in a cyclohexane matrix. Carbazale in THF exhibits a broad phosphorescence emission but this is still displaced to the blue in comparison with the quasi-linear emission in n-heptane.

The shifts observed to the blue in the phosphorescence emission in THF are unexpected if compared with the behaviour in glassy matrices. According to Zander[216], $\pi - \pi^*$ phosphorescence which is usually found in hydrocarbons is slightly dependent on the solvent and in the majority of the cases only a small red shift is observed by changing from a non-polar solvent (3-methyl-pentane or various mixtures of methylcyclohexane: isopentane between 1:5 and 5:1 v/v) to polar solvents such as EPA or a mixture of ether and ethanol (1:2 v/v).

In frozen polycrystalline matrices such as the n-paraffins, shifts have also been observed when changing from one member of the family to another. Laipanov[217] observed that the phosphorescence emission of

5,6-benzoquinoline was displaced to higher frequencies (blue shift) when the molecule was in an odd C-number n-paraffin matrix; differences between the even C-number member and the adjacent odd member were in the order of 40-50 cm^{-1}. Laipanov attributed such shifts to the interaction forces of the terminal groups on the excited molecule and a possible relation between this effect and the different physicochemical properties of the odd and the even C-number members of the n-paraffin family.

Effect of impurities in THF

We have observed during this work that when an old batch of THF was used as solvent, coronene at concentrations lower than 1×10^{-5} M failed to produce quasi-linear emission; in its place appeared a broad band emission displaced ca. 30 cm^{-1} to the red from the quasi-linear emission maxima at 424.85 or 443.65 nm.

At concentrations higher than 1×10^{-5} M quasi-linear luminescence was observed together with this broad emission. Determination of the peroxide content (using the iodometric method proposed by Liebhafsky and Sharkey[218]) of the THF gave a value of 0.5% (as the most probable present hydroperoxide). Treatment of THF with Na-wire and redistillation produced a solvent with a very low peroxide content (0.020%) and in this case the coronene gave rise to narrow quasi-linear emission on which a very weak shoulder was superimposed. Fig. VIII - 14 shows the effect of the peroxide content in the coronene emission.

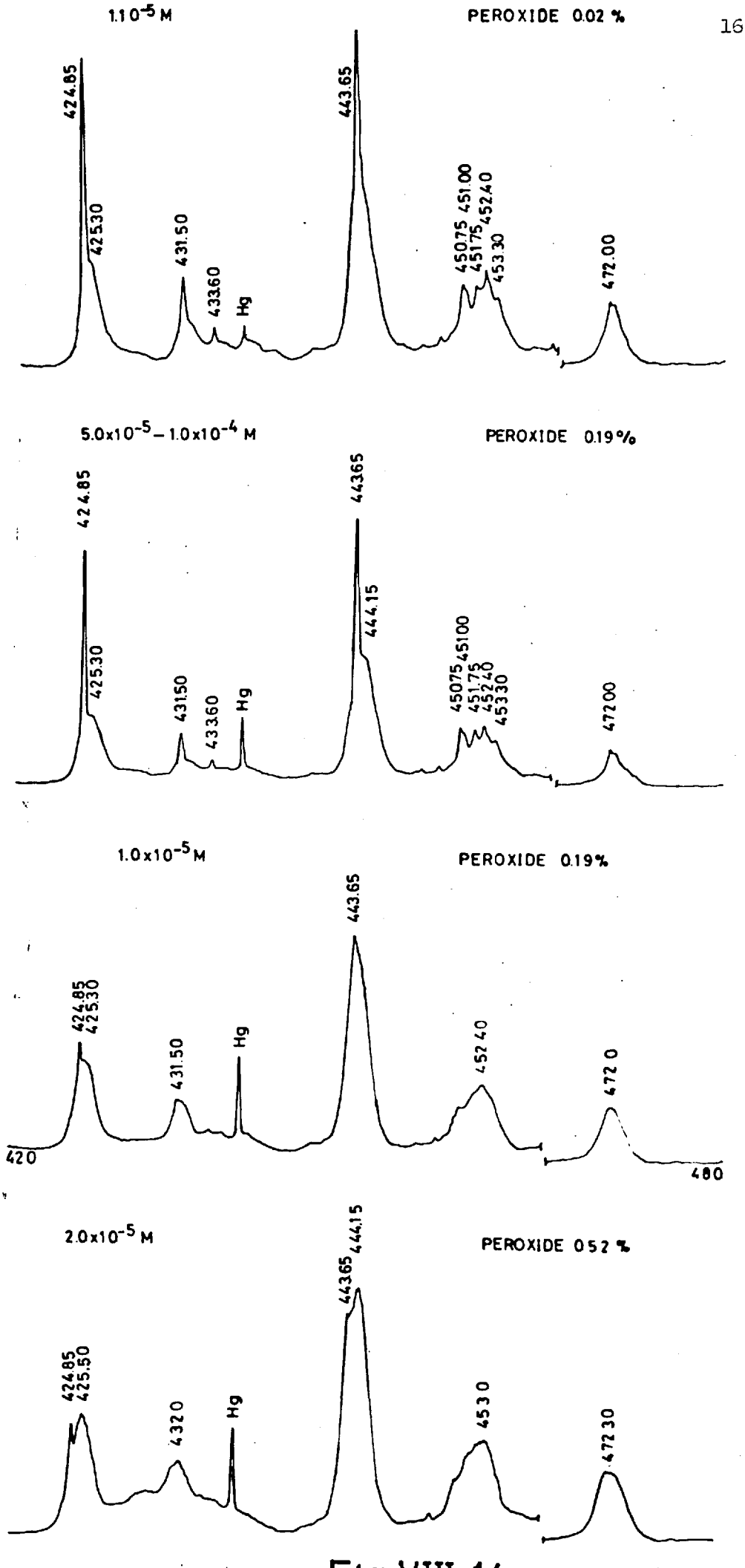
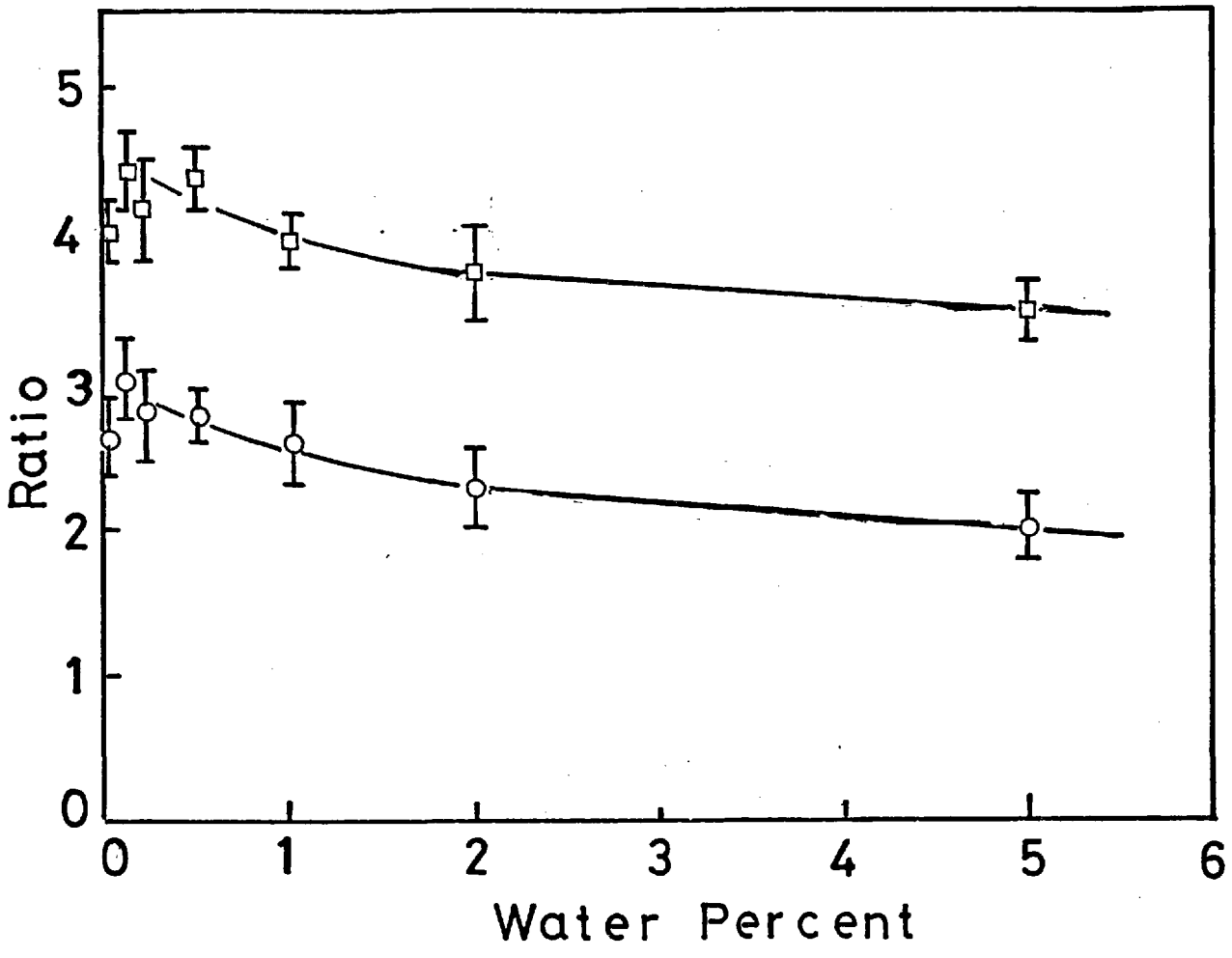

1.10⁻⁵ M
PEROXIDE 0.02 %
424.85
425.30
431.50
433.60
Hg
443.65
450.75
451.00
451.75
452.40
453.30
472.00
5.0x10⁻⁵ – 1.0x10⁻⁴ M
PEROXIDE 0.19%
424.85
425.30
431.50
433.60
Hg
443.65
444.15
450.75
451.00
451.75
452.40
453.30
472.00
1.0x10⁻⁵ M
PEROXIDE 0.19%
424.85
425.30
431.50
Hg
443.65
452.40
472.0
420
480
2.0x10⁻⁵ M
PEROXIDE 0.52 %
424.85
425.50
432.0
Hg
443.65
444.15
453.0
472.30

Fig.VIII-14



EFFECT OF WATER IN THE EMISSION OF CORONENE IN TETRAHYDROFURAN

□ Ratio $I_{424.85}/I_{425.30}$

○ Ratio $I_{443.65}/I_{444.15}$

Fig. VIII-15

The addition of oxygen to the purified THF did not produce any effect. Low concentrations of water (0.1 - 0.2%; determined using the Karl-Fischer method[219]) appear to be beneficial to the quasi-linear emission intensity. At concentrations of water higher than 0.2% the quasi-linear emission begins to be suppressed and the ratio of the quasi-linear emission intensity to that of the weak shoulder emission decreases (see Fig. VIII-15). Apparently both water at higher concentration and peroxide destroy the conditions within the matrix which are responsible for the production of the quasi-linear emission. The presence of peroxide has a more drastic quenching effect than water and can result in complete annihilation of the quasi-linear emission, when only low concentrations of coronene are present. The

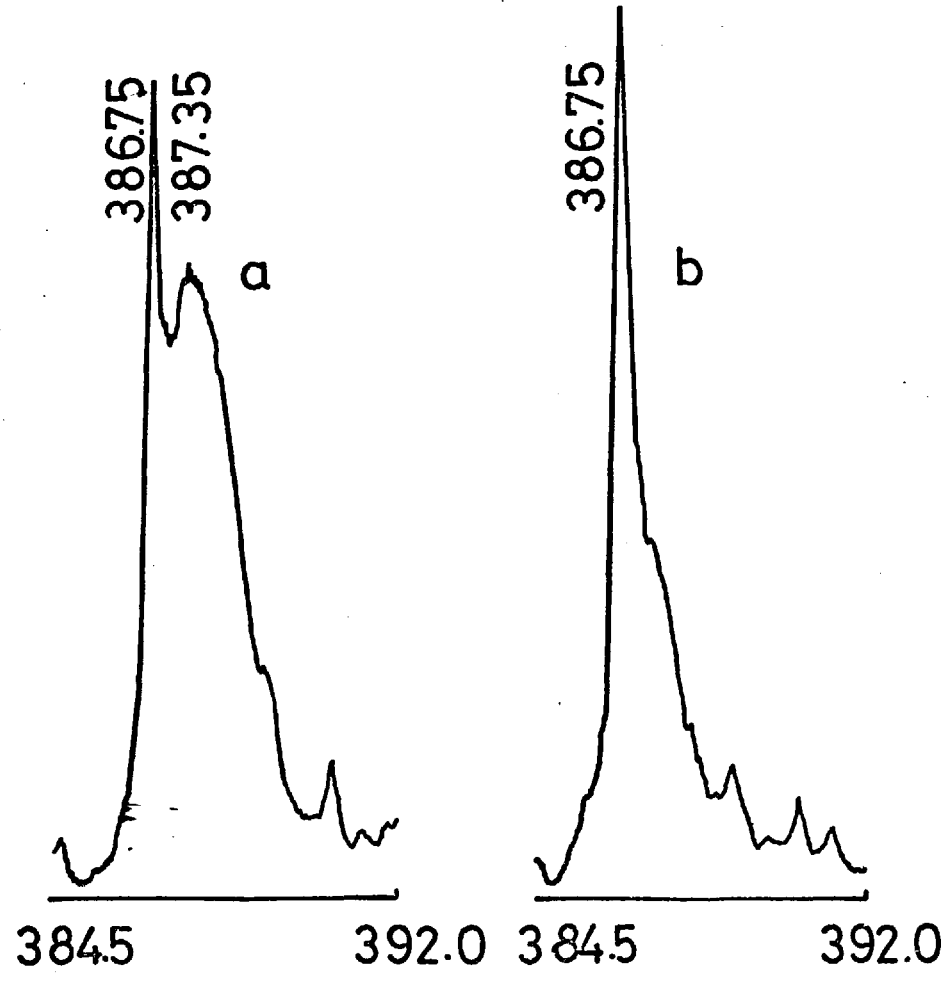


Fig. VIII - 16

same effect of peroxide was observed with 1,2-benzopyrene; a broad peak is observed, shifted 42 cm^{-1} to the red (a in Figure VIII-16) when THF with 0.5% peroxide is used in relation to the emission in THF matrix with 0.02% (b in Figure VIII-16).

The formation of a complex between the peroxide and the analyte molecule is possible; Ladner and Becker[220] observed a similar effect in the luminescence, at 77K, of acridine in the presence of aged diethyl ether and speculated on the possibility of the formation of a peroxide complex with the solute.

Another probable effect of the presence of peroxide in the old THF is the modification of the emission when a solution is aged; most of the compounds show a different emission, (e.g. phenanthene, chrysene, 3,4,9,10-dibenzopyrene, etc.) shifted to the blue, usually broader - with 3,4,9,10-dibenzopyrene, however, the modified compound presented quasilinear emission.

The modifications and shift to the blue is probably due to the attack (peroxidation) on the emitting aromatic molecule.

Chapter IX

Systems of Electronic Signal Processing Applied to Quasi-Linear Spectrometry

Introduction

As described in Chapter III photographic or photometric methods of detection have usually been employed by researchers in the fundamental studies or the analytical application of quasi-linear luminescence. Although the photographic method has the advantage of being an integration method, so that very low light intensities can be detected, it has the disadvantages of being time consuming, and needs constant calibration and is subject to a serious limitation in the non-linearity of the photographic emulsion with respect to the amount of radiant energy received. Photomultiplier detectors used with a voltmeter or a potentiometric recorder are the most conventional devices used for detecting the signals in the range from UV to the near infra-red. It is usual in such cases, to pass the anode photocurrent pulses across a load resistor to generate a voltage signal which is then measured. Between the read-out device a d.c. amplification stage is usually employed; this is the most simple and least expensive type of amplification. The electrical noise band width, Δf, of the amplifier is determined by the amplifier time constant τ, which is determined by the resistor-capacitor product (RC) somewhere in the circuit of the d.c. amplifier and is expressed by the relationship[179]

$$\Delta f = (4\tau)^{-1} = (4RC)^{-1} \qquad \text{IX - A}$$

The great disadvantage of this type of amplifier is that it amplifies undesirable d.c. signals such as the dark current, the back-

ground current and the noise, etc. - that are normally present with the signal of interest. A backing-off device is normally used to electrically balance some of this unwanted signal. Drift is another problem which results in the necessity of a frequent change in the backing-off adjustment. Some of the noise can be attenuated by changing the RC product; a compromise between the response time and the velocity of scanning must be established, however, as sometimes the phenomenon is of short duration or long response time can distort the peak being recorded. Noise, however, is still observed in the recorded spectrum at very high sensitivity settings and it is this noise which establishes the limit at which a signal can be observed or resolved. A photomutiplier and d.c. amplification system together with a potentiometric recorder has been used in the present work as already described in Chapters IV and V.

In constrast with atomic emission from flame or non-flame devices, where the phenomenon is time limited by the amount of sample, by the flicker noise or by the nature of the volatilisation process, molecular luminescence spectrometry in solution at room temperature or in frozen matrices (glassy or polycrystalline) at low temperature is a steady phenomenon (an exception may be made when a photochemical reaction occurs with a consequent decrease in the luminescence intensity or the formation of another luminescing species) with considerable smaller problems of detection. Due to this, molecular luminescence systems seem to be the obvious choice for the use of a read out time dependent devices such as constant time (or constant charge), photon counting or signal averaging. Either higher sensitivity or higher resolution is expected to be observed during the use of such devices.

1. Constant time and constant charge systems

One possibility for processing an electronic signal is by the use of a d.c. integrator. A d.c. integrator is a circuit whose output voltage, e_o, is proportional to the time integral of the input voltage[179]

$$e_o = K\int_o^{t_i} e_i dt \qquad \text{IX - B}$$

where K is a circuit constant and $e_o = 0$ at $t = 0$. If t_i is made equal to K^{-1} then[179]

$$e_o = \frac{1}{t_i}\int_o^{t_i} e_i dt = \bar{e}_i \qquad \text{IX - C}$$

where e_o is the mean value of e_i during the interval $t = 0$ to $t = t_i$. Such a circuit is called a constant time integrator and can be very easily constructed from an operational amplifier with a capacitor in the feedback loop.

Another possibility is to fix a value of the capacitor charge as the constant, so that time becomes the variable quantity. In this case we have a constant charge integrator and a reference signal must be introduced in the circuit; this is normally done by using a second photomultiplier which detects a small fraction of the overall input signal.

Application of d.c. integration systems

Constant time, and to a lesser extent constant charge systems, are already available in commercial instruments either for atomic emission (Applied Research Laboratories)[221] atomic absorption (Shandon Southern)[222] or molecular luminescence (American Instrument Co.)[223].

Aldous, Dagnall, Sharp and West[224] applied integration to weak and transient signals common to non-flame methods of atomisation. Lukasiewicz and Fitzgerald[225] applied with success the constant time integration method to low resolution molecular luminescence; tryptophan fluorescence was measured with better precision and 100-fold better sensitivity in comparison with the direct analogue d.c. readout method.

Experiments with d.c. integration

For the experiments with constant time and constant charge integration a Quantoscan spectrometer (Applied Research Laboratories, Ltd. Luton), which is an instrument designed for elemental analysis by spark emission spectrometry, was employed. This was modified for low temperature work by removal of the spark excitation stand and coupling with a cell and excitation system similar to one described in Chapter V in which a sample holder, an excitation source (Philips MBW/U; 125W), lenses and an interference filter and excitation with an angle of ca. 60^{o} was used; an illumination of 45^{o} or less was not possible due to the physical construction of the monochromator. The measurement and control units provided with the instrument were used; in these units were located the constant time integration system (with a built-in electronic timer) and the constant charge integration system.

The monochromator of the Quantoscan is a one meter, Czerny-Turner, stigmatic mounting, utilising a plane grating (with dimensions of 50 x 25 mm) blazed at 300.0 nm, spectral range 190 - 880 nm and with a reciprocal linear dispersion of 0.8 nm mm^{-1}[221]. The aperture is not specified by the manufacturer. If estimated from the focal length and

the grating size, we obtain the relatively low aperture of f/25. The rotation of the grating is made by a precision screw drive and by a motor together with a 10-speed gear box. A scanning velocity of 4.0 nm min^{-1} was used in all experiments. The slits are not adjusted from outside and a travelling microscope was used when re-adjustment of the slit widths was necessary.

In the measuring system a galvanometer can be used for profiling and analysis. An output of the amplifier to a recorder was used. A Servoscribe (Model RE 511.20) recorder was employed. In the instrumental system there exists the possibility of choosing between constant time or constant charge integration mode. When in the constant charge mode a second (reference) photomultiplier is in use; the light beam originating from the sample is partially reflected in a quartz fibre which is used as a reference beam. Both reference and measuring photomultipliers were side-on Hamamatsu R-106 (response type S-19)[226] with a spectral response range of 160 to 650 nm. Both photomultipliers are driven by an EHT power supply in the console and there is a possibility to choose the applied voltage from 560 to 700 V in steps of 20 volts. The silica tube-Dewar accessory described in Chapter VII, was used in most of the experiments concerned with constant time and constant charge.

A study using 3,4-benzopyrene in n-octane was made to compare the analogue output from this instrumental arrangement with the constant time and constant charge integration modes. The analogue output was indeed very weak, even in comparison with the preliminary arrangement described in Chapter IV, probably due to factors such as the high f-number and the type of photomultiplier used. With the recommended slit width (0.02 mm) no signal was isolated from the noise. Larger slits

width was then used, normally 0.2 mm.

a. Constant time

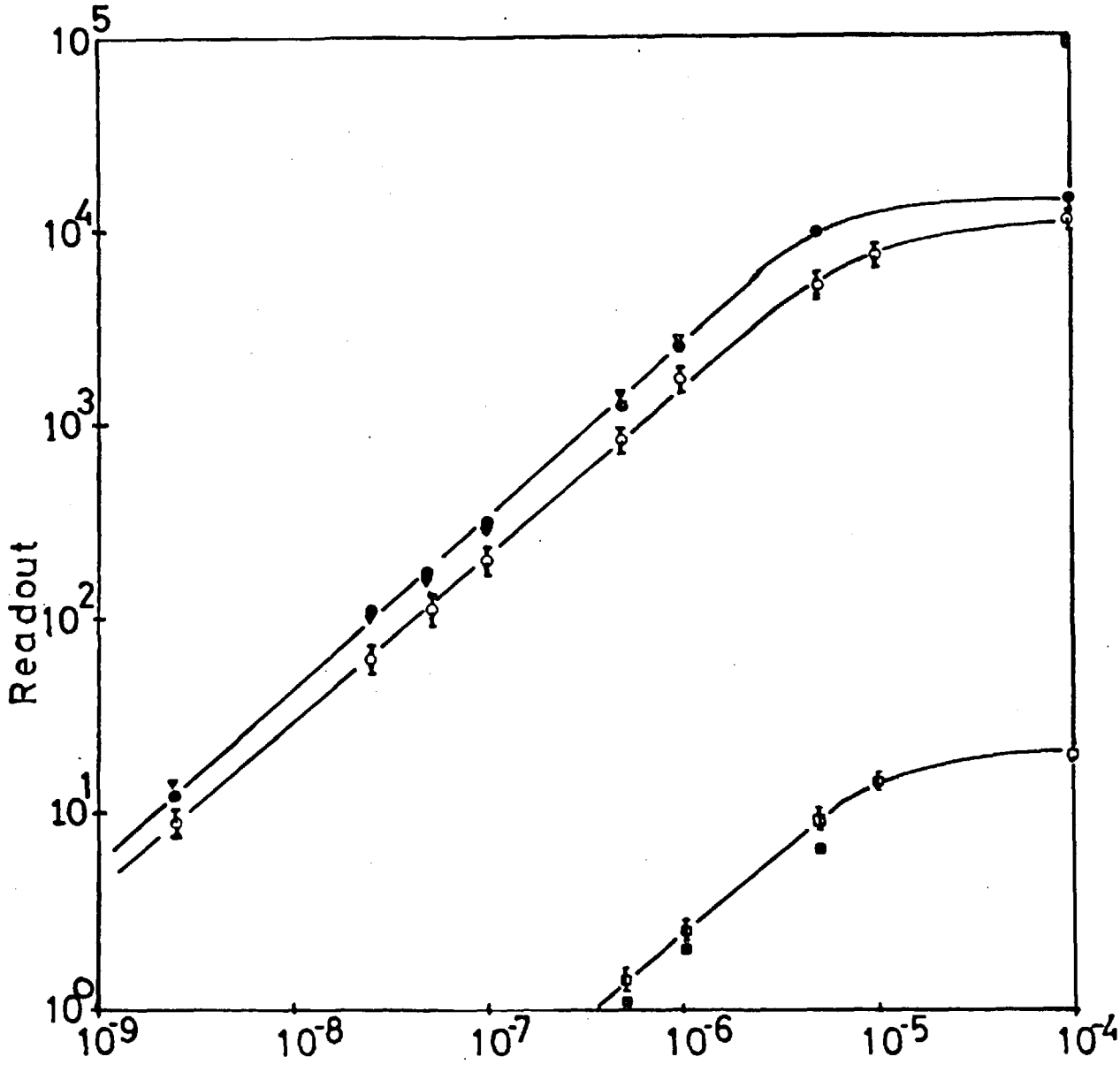
During the constant time measurements difficulties were observed in the reproducibility of the data obtained, due to change in the position of the tube. A relative standard deviation of 10 - 20% was then observed using a 3,4-benzopyrene with a concentration of 5×10^{-6} M.

To improve the situation attempts were made by using a spinning device similar to one proposed by Hollifield and Winefordner[184]. A mini-stirrer motor (Electrothermal Ltd.) was used to rotate the sample tube. The motor axle and the sample tube were coupled by a rubber sleeve. A relative standard deviation (using a 5×10^{-6} M 3,4-benzopyrene solution) of <10% was obtained.

The observed background was quite large, and as a backing-off unit was already provided in the instrument the integrated signal was previously zeroed at 400 nm between measurements. An integration time of 60 seconds was used in the experiments and 700 V was applied to the photomultiplier.

Figure IX-1 shows the results obtained. An improvement of 3 orders of magnitude was observed if the constant time mode is compared with the analogue mode provided in the instrument. The relative standard deviations (RSD) presented in Table IX-1 show that better precision is obtained with the constant time integration system in comparison with the analogue system. The limit of detection with the analogue system was ca 5×10^{-7} M and with d.c. integration 1×10^{-9} M.

Fig. IX-1



•-Constant time (60 sec.) Appl. volt: 700 V, spin.
▾-Constant charge, Appl. voltages: Photo. meas. 700V; Photo. ref. = 680 V, spin.
◦-Constant time (60 sec.), without spin.
▫,▪-Analog read.; without (▫) and with spin. (▪).

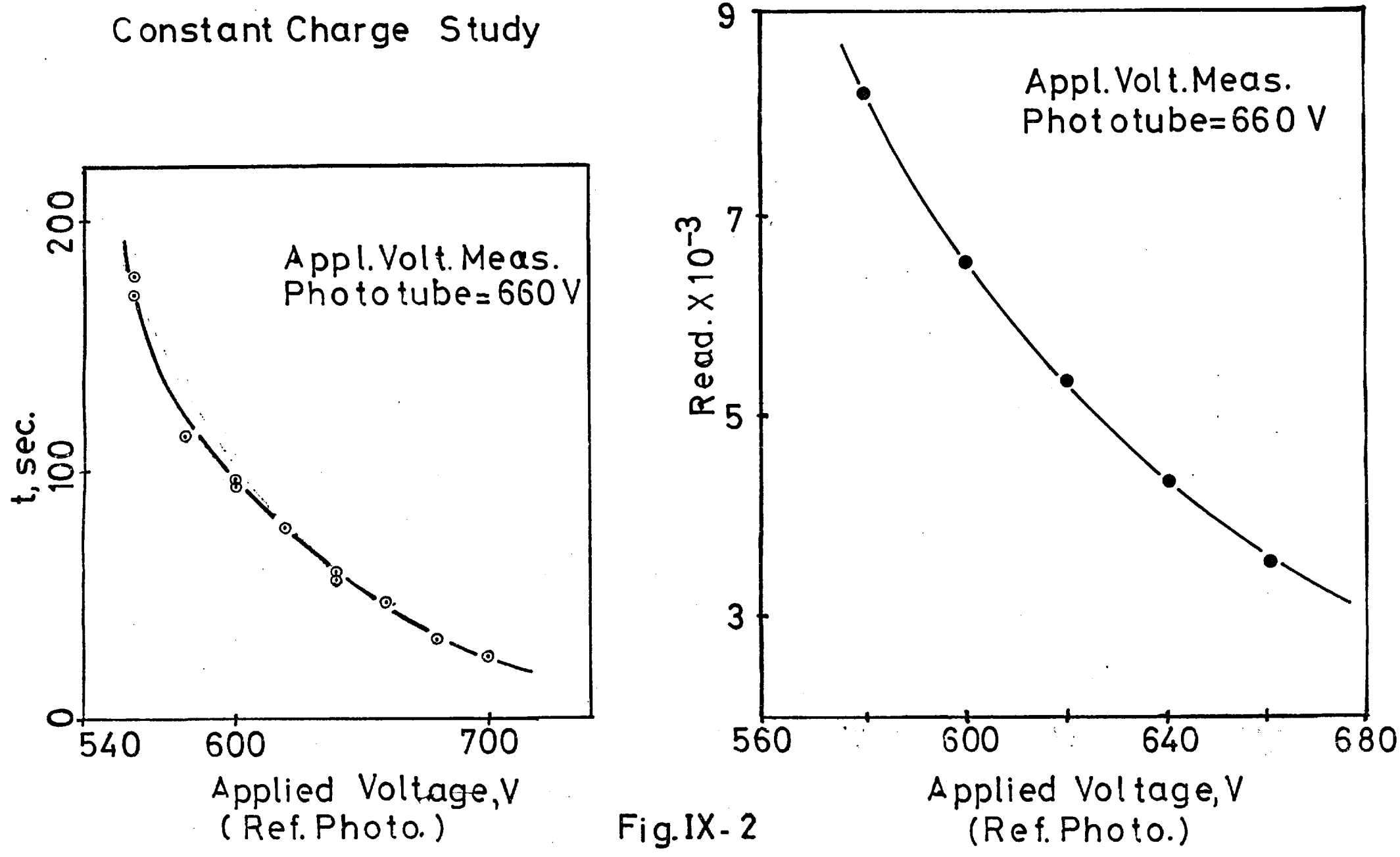

Constant Charge Study
Appl. Volt. Meas.
Phototube=660 V
t, sec.
0
100
200
540
600
700
Applied Voltage, V
(Ref. Photo.)
Appl. Volt. Meas.
Phototube=660 V
Read. X10⁻³
3
5
7
9
560
600
640
680
Applied Voltage, V
(Ref. Photo.)

Fig. IX-2

TABLE IX-1

Concentration (M)	2.5×10^{-9}	2.5×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	5×10^{-6}	1×10^{-5}	1×10^{-4}
Analogue RSD,%	-	-	-	-	~25.0	9.0	3.0	2.5
Constant time RSD,%	4.4	6.0	5.5	9.4	3.0	4.6	3.0	3.0
Constant charge RSD,%	6.0	4.4	1.6	0.7	1.4	0.9	-	-

b. Constant charge

With constant charge another parameter needs to be adjusted; this is the gain of the reference photomultiplier. As can be seen from Figure IX-2 the higher the voltage applied to the reference photmuliplier the less time is needed for the integration, maintaining the light flux through the optical system constant. As the time of integration and the data integrated are directly proportional, the same curve shape is obtained (Figure IX-2). For the purposes of comparison the applied voltage was choosen so that the time of integration was ca. 60 seconds. The curve of signal read-out vs concentration (Figure IX-1) obtained using constant charge has the same appearance as the constant time curve. Also the limit of detection is roughly the same. Table IX-1 shows, however, that lower relative standard deviations are obtained with the constant charge mode of operation. This improvement in precision can be explained as due

to the presence of the reference photomultiplier in the system; variations in the overall light input due to excitation light fluctuations or variations in the tube position(although the sample tube was spinned) are thus corrected by the second photomultiplier with a consequent increase or decrease in the time of integration.

2. Photon counting

Another signal collection and processing technique available is photon counting. Basically the photon counting technique involves the direct digital recording of the current pulses resulting at the anode of a photomultiplier, caused by the photon impact in the photo-cathode, and if the electronic resolution is sufficient the measurement provides a direct digital read-out of the radiation intensity.

According to Franklin, Horlick and Malmstadt[227] the advantages of photon counting are direct digital processing, a decrease of effective dark current by orders of magnitude, improvement of signal-to-noise ratio (SNR), accurate signal integration, improved precision of analytical results and elimination of reading error. The ability to detect low light levels provides the possibility of operation at narrow spectral band pass with consequent improvement in spectral resolution. Furthermore the direct digital output obtained in photon counting makes it ideal for interfacing with modern computerised data handling systems[228].

Principles of photon counting

Much has been written about photon counting[227-31] andthe brief

review of its principles given here will be as concise as possible.

The production of photoelectrons, by the incoming photon impinging in the photo cathode surface, depends on the quantum efficiency of this surface and the efficiency is dependent on the wavelength. Secondary electron emission occurs inside the photomultiplier in the dynode chain which is how the amplification takes place[157]. Typical photomultipliers have from 9-20 dynodes providing gains in the range of 10^5-10^8. The gain is also critically dependent on the voltage applied across the dynode chain. The power supply voltage fluctuation is important to the photon counting method as the fluctuation, although it does not affect the count number, will affect the pulse amplitude. When pulse height discrimination is employed the supply voltage fluctuation will thus affect the amplitude distribution[227]. If the gain is high enough to permit virtually every charge pulse from a signal photon event to be counted, i.e. if the signal pulses have a narrow pulse height distribution and are much larger than the background noise pulses, the signal count rate becomes less dependent on photomultiplier gain or photomultiplier EHT supply stability and also on the discriminator level stability.

A pulse height distribution is normally present as each photoelectron amplified does not produce an identical current pulse at the anode. This distribution is a consequence of the statistical nature of secondary electron emission. The current pulses that appear as a result of amplification in the tube, have a pulse height distribution closely approximated by a Poisson distribution[227-8,232].

Franklin, Horlick and Malmstadt[227] have also observed that the pulse height spectrum of the dark current is different from that of the signal pulses and have concluded that the dark current is not Poisson in

nature. The same workers have analysed the sources of dark current. These are summarized below.

Sources of dark current

A photomultiplier in complete darkness still produces current pulses. Causes of this dark current can be thermionic emission, cold field emission, radioactivity, gas ionization and interelectrode ohmic leakage. The first three of these are pulsed in nature.

The major contribution to the dark current is the thermionic emission (or also called thermal dark current) and obeys Richardson's law[233].

$$I = 1.2 \times 10^{2} \times T^{2} \, e^{-\left(\frac{1.16 \times 10^{4} \times \phi_{T}}{T}\right)} \qquad \text{IX - D}$$

where T is the temperature and ϕ_T is the thermal work function. The current originating from the thermionic emission is, as can be seen from equation IX-D, extremely dependent on the temperature; cooling to about 253 to 233 K is sufficient to reduce this effect dramatically[233]. Usually for each 10 K decrease in temperature the thermionic emission decreases to half. For each photomultiplier there is a minimum optimum temperature; with a Cs - Sb type cathode ca. 253 K is the optimum temperature. Liquid nitrogen, dry ice or thermoelectric cooling chambers have been used for such purpose. Also, specially made photomultipliers having a higher than usual thermal work function threshold, can be used to give rise to a very low thermionic emission - e.g. EMI type S; these may be used without cooling[228].

Cold field emission is not an important source of dark current

except when the PMT is run at higher voltages than those recommended. Radioactive sources produce a small current. The usual source is the presence of natural K^{40} isotope in the glass used in the PMT envelope construction and which emits β particles with the production of Čerenkov photons from the glass. Cosmic particles and other sources of radioactive material in the vicinity of the PMT can also cause the glass to fluoresce. The ohmic leakage is a small d.c. signal which always exists in a PMT and, being of unpulsed nature, does not contribute to photon counting measurements.

The dark current is also function of the applied voltage and, as stated above, at higher voltage cold field emission could result. The choice of the best applied voltage will be discussed later.

Pulse pile-up (coincidence) effect

Pulse pile-up is a term given to the situation that occurs when the photo-electron pulses are not fully time resolved by the counting circuitry. According to Sharp[228], two pulse pile-up mechanisms can occur; in the first, two pulses overlap and are counted as one, a frequent fact at high photon arrival rates. The other mechanism is the addition of two small dark current pulses that add algebraically and the resultant large pulse is counted, although a discriminator (a 'window') is in use to cut the dark current pulses of different pulse amplitude.

Signal to noise ratio (SNR)

The signal or the dark and background currents do not, in general, constitute a smooth d.c. current but constitute also an a.c. or noise component superimposed ontit. This undesirable component creates a limit at which the minimum level of signal can be isolated, identified and the measurement can be made. The primary source of noise in a photomultiplier at room temperature is usually called 'shot noise' or photon noise; this noise is due to the random emission from the cathode and the random arrival at the anode, even when the incident light flux is constant; the same occurs with the dark current from thermionic emission.

Other sources of noise are described as the Johnson noise and the flicker noise. Johnson noise is the noise caused by the electrons moving through a conductor which continually exchange energy with thermal vibrations of the lattice ions, resulting in random fluctuations in the charge density and consequent variations in the potential drop across the conductor. The Johnson noise obeys the following relationship[234]

$$E = (4RkT\Delta F)^{\frac{1}{2}} \qquad \text{IX - E}$$

where E is the root-mean-square (rms) noise voltage contained in a band width ΔF produced in a registor of resistance R, k is the Boltzmann constant, and T is the resistor temperature in K. The Johnson noise is, however, of much less influence in comparison with the shot and flicker noise[228].

The flicker noise is normally caused by fluctuation in the experimental arrangement such as in the excitation source radiance (due to line voltage variations, lamp aging, arc wander, thermal turbulence

in the sample cell (coolant convection, bubbling and snow particles - principally in low temperature luminescence spectrometry) and some of the photomultiplier noise and amplifier noise - the last two of small magnitude[179].

From the point of view of frequency characteristics, noise has no characteristic periodic character. To characterize noise it is more desirable to analyse the frequency components in the noise waveform as the noise-power spectrum - which is a plot of the noise power as a function of frequency[234] (Fig. IX - 3).

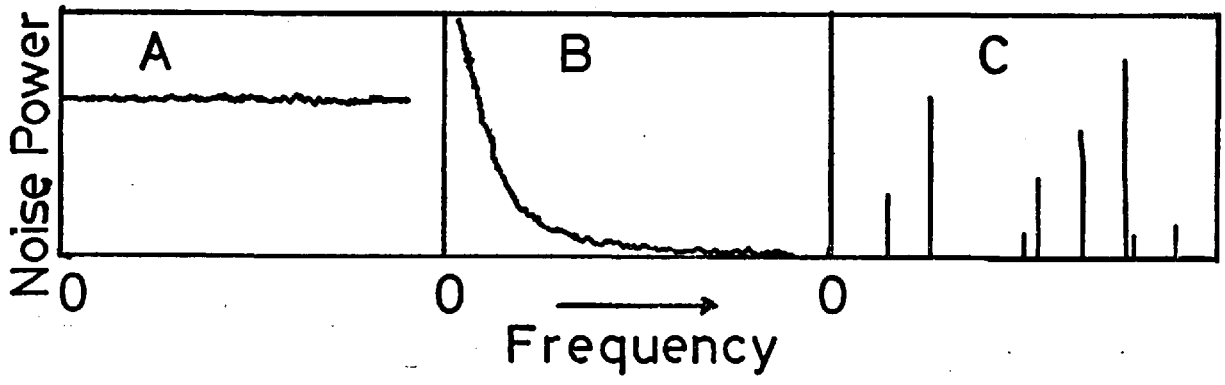


Fig. IX - 3 - Noise power spectra. A - 'White noise', B - 1/f noise, C - Interference noise (From reference 234)

The white noise (Fig. IX - 3A) is characterized by a constant spectral power density, independent of the frequency - e.g. shot noise and Johnson noise which are random in nature have such spectral characteristics. Figure IX-3,B shows the power spectrum of flicker noise which is characterized by a noise poise nearly proportional to the inverse of the frequency and for that reason is sometimes called 1/f noise.

Interference noise (Figure IX-3C) is the type of noise which

originated from mains, high voltage spark sources, radio trasmitters, microwaves generators, etc.[234].

Hieftje[234] has also suggested that other types of noise may be present such as impulse noise (as from the start-up of large motors or a lightning flash) which has a broad frequency spectrum, and distortion noise - e.g. noise from transmission losses, reflection and the interaction of the signal with various disturbing elements.

Shot noise normally obeys Poisson statistics and is sometimes called Poisson noise. The Poisson noise can be determined by counting statistics as the standard deviation of a measurement or the square root of the number of counts and is given by[179]

$$\sigma_c = \sqrt{C_T} \qquad \text{IX - F}$$

where σ_c is the standard deviation and C_T is the total counts accumulated per time T. This means that the more counts recorded for a given measurement time the better the precision. The SNR for a specific measurement can be estimated by dividing the count by the square root of the count.

$$S/N = \frac{C_T}{\sqrt{C_T}} \qquad \text{IX - G}$$

However, most experiments are made in a situation such that unwanted background pulses are present. In these cases the SNR is more complicated, and the background count must be substracted from the measured background plus signal count[227,229].

$$S/N = \frac{(R_s T)^{1/2}}{(1 + 2R_B/R_s)^{1/2}} \qquad \text{IX - H}$$

where R_s is the signal count rate, R_B is the background count rate and T has the same value as for equation IX-F. If background is small in comparison to the signal count rate the equation can be written

$$S/N = (R_s T)^{1/2} \qquad \text{IX - I}$$

If the background is large the equation IX-H becomes[227,229]

$$S/N = \frac{(R_s T)^{1/2}}{(2R_B/R_s)^{1/2}} = \frac{R_s T^{1/2}}{(2R_B)^{1/2}} \qquad \text{IX - J}$$

Applications of photon counting

The use of photon counting for analytical applications has been growing in recent years. Piepmeier, Braun and Rhodes[235] and Franklin, Horlick and Malmstadt[227] have discussed the advantages and practical considerations of the application of photon counting to quantitative analysis, in particular to solution absorption spectrophotometry. Alger, Dagnall, Sharp and West[236] proposed the use of photon counting in conventional flame and non-flame atomic absorption; an instrument was built and evaluated in comparison with a d.c. amplifier-integrator.

Aoshima, Iriyama and Asai[237] have applied photon counting to molecular fluorescence using a filter fluorimeter. Linearity was observed for riboflavin from 4×10^{-5} μg ml^{-1} to 1 μg ml^{-1} in aqueous solution. The results showed that the apparatus had a 40-fold higher sensitivity than a typical commercial spectrofluorimeter. Chlorophyll *a* and chlorophyll *b* were also studied. Curry, Pardue, Mieling and Santini[238] constructed a filter fluorimeter in which photon counting was utilised for the detection of fluorescence. The instrument was evaluated

and the detection limit obtained for fluorescein was improved by a factor of 3 compared to that obtained using a conventional spectrofluorimeter. For Rhodamine B, the detection limit was improved by a factor of 4.5.

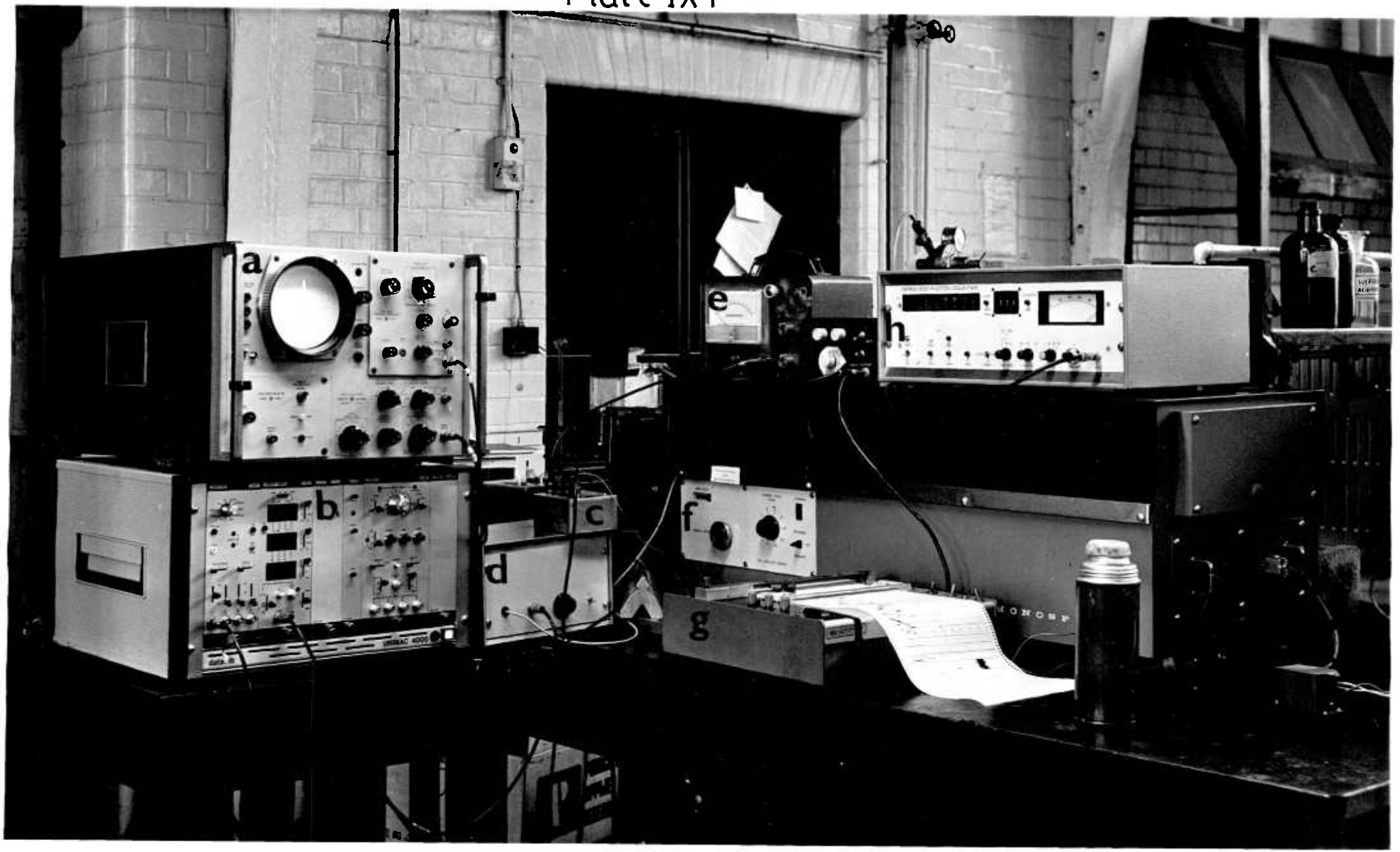
Tuan and Wild[239] have utilized photon counting with a high resolution spectrometer; the scanning was made using a step motor and the data was recorded digitally in the form of listings or punched tape. This assembly has been successfully used in basic research in the Shpol'skii effect.

Photon counting has been also applied to atomic fluorescence spectrometry[230,240]; the photon counting system was found to be superior, when using low intensity sources, in comparison with a lock-in amplification system[240].

Photon Counting Experiments

In the present work we have studied 3,4-benzopyrene and 3,4,9,10-dibenzopyrene using a photon counting system coupled to the monochromator (Monospek 1000) and photomultiplier already described in Chapter V (see also Plate IX - 1, f). During the preliminary experiments the silica tube - Dewar assembly and an EMI 9601B photomultiplier were employed; this arrangement gave very high dark current counts, even after cooling the photomultiplier housing with dry ice, (ca 1300 counts sec^{-1}). Later an EMI 6256S tube, which is characterized by a very low dark counts (at ambient temperature ca 40 counts sec^{-1}; dry ice temperature ca 4 counts

Plate IX-1



sec^{-1}) was used. The copper cryostat described in Chapter VIII was used in most of the experiments. An EDT Supplies Ltd., Series 300 photon counter was used; this instrument has a capacity of 10^7 minus one counts. (Plate IX-1, h).

For the analogue readings the signal was amplified using a micro-ammeter (RCA, Model WV-84C) as amplifier; the amplified signal was then introduced into a recorder (Servoscribe RE 5 11.20). N-Octane was used as solvent and was purified as described in Chapter V.

A specially built cooling chamber, with a slightly modified design to that used by Sharp[228] was used to cool the photomultiplier during the experiments. A constant flow of dry nitrogen was introduced around the photomultiplier itself, to avoid condensation, either during the cooling or the warming cycle.

Determination of experimental parameters.

Photon counting parameters were first established to optimise the operation. By cooling the photomultiplier the expected decrease in dark counts was observed.

In contrast to right angle illumination and using clean solution at room temperature, with frontal illumination and frozen polycrystalline matrices a large amount of scattered and reflected excitation light is observed. This scattered and reflected light is inevitably introduced into the optical system of the detector. With a d.c. amplification or d.c. integration system this unwanted light can usually be backed-off; in our case, however, this was not possible using the photon counting

technique. In such conditions where a small signal is to be detected in the presence of a large background a synchronous photon counting system, when two simultaneous channels of count information is accumulated and then processed[230], is required. In our case the background counts at 400.0 nm (with the photomultiplier cooled) were of the order of 600 - 800 $csec^{-1}$, which suggests that cooling is unnecessary when the low dark current photomultiplier (EMI 6256S) is employed. Further indication of the utility of cooling was made by the determination of the relative standard deviation of a signal from a 3,4-benzopyrene solution with a concentration of 1×10^{-9} M. Table IX - 2 shows that no appreciable difference is observed between ambient temperature operation and dry ice temperature operation.

TABLE IX - 2

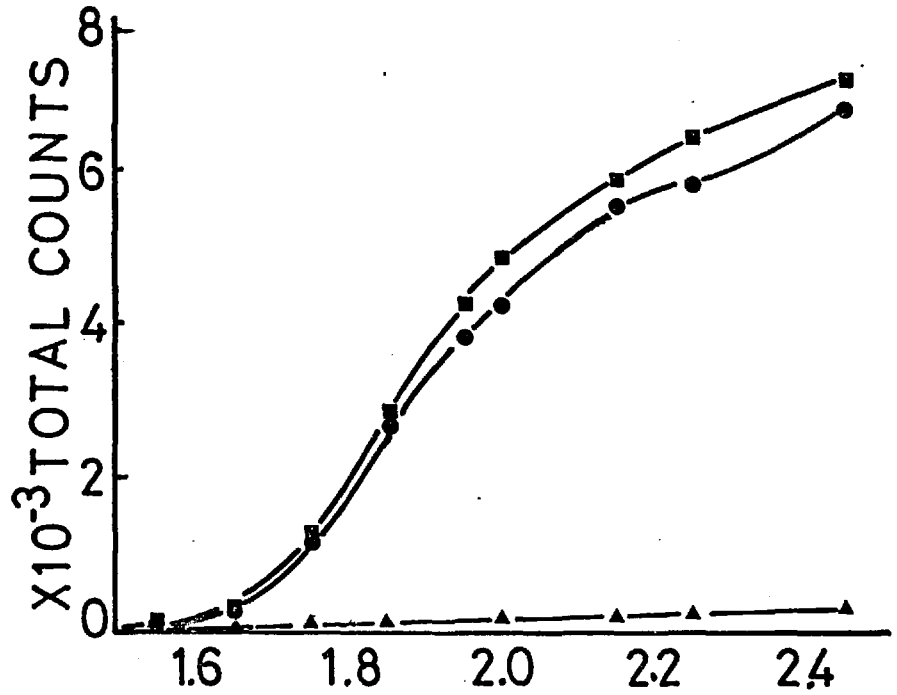
Condition	Relative Standard Deviation			
Room temp.	1.90	2.38	2.23	(2.17)
Dry ice temp.	2.23	2.09	1.91	(2.08)

(Average values in parenthesis)

Fig. IX - 4 shows the variation of total counts (in 5 seconds) using the quasi-linear emission of 3,4-benzopyrene in n-octane, (1×10^{-9}M) *versus* the photomultiplier applied voltage. The signal counts were taken at the 403.0 nm peak and the background was counted at 400.0 nm. The figure shows the strong influence of the background in the measurement, in particular at lower concentrations (1×10^{-9}M).

In figure IX - 5 the variation of the signal to noise ratio is

Fig. IX-4



■ TOTAL SIGNAL. ▲ DARK CURRENT

● BACK GROUND

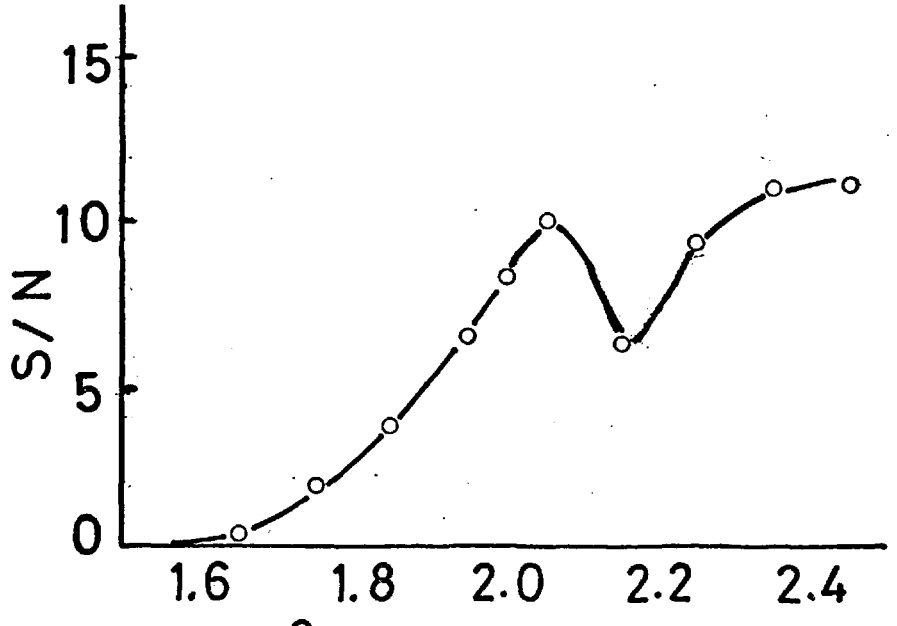


Fig. IX-5

shown (using the formula for high background IX - J), as a function of the applied voltage; the photomultiplier was cooled with dry ice. At 2200 V a sharp decrease in the SNR is observed. With the photomultiplier at ambient temperature the SNR at 2000 - 2050 V shows almost the same maximum, but the decrease at 2200 is slightly larger. There is no immediate possible explanation for this effect of the decrease in the SNR, at a particular voltage.

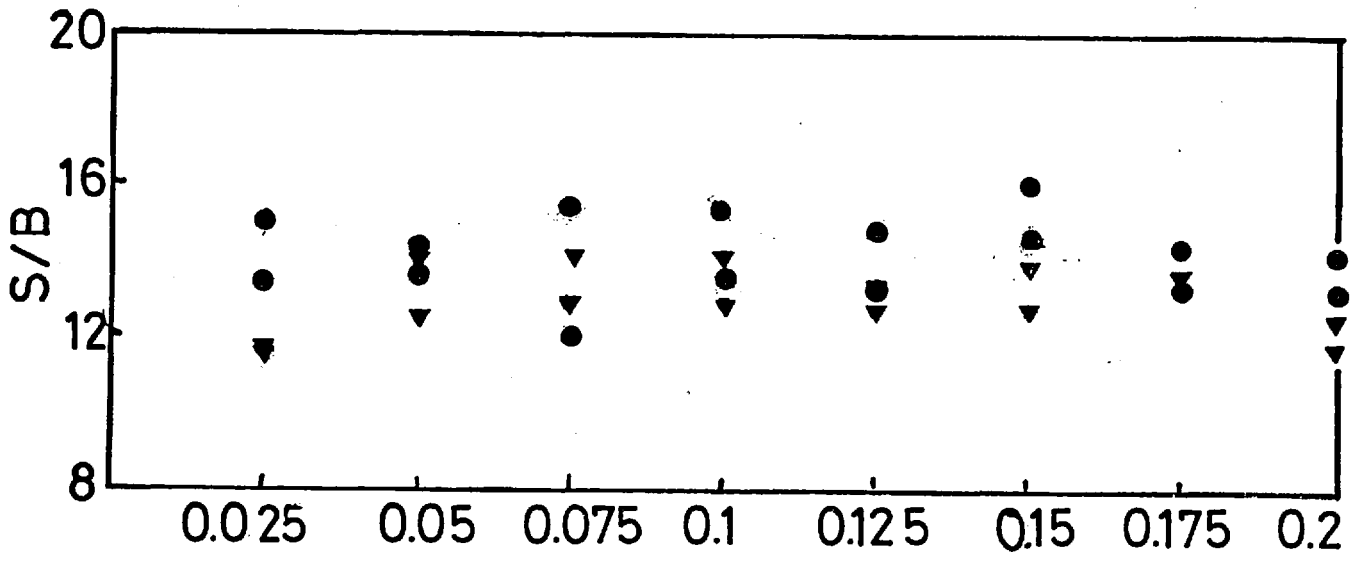


Fig.IX-6

The influence of slit width on the signal to background (S/B) ratio obtained was studied; the results were plotted and are shown in figure IX - 6. Both analogue and photon counting signals were compared using 1×10^{-7} M 3,4-benzopyrene. Between 0.050 mm to 0.175 nm the signal-

background ratio (using photon counting) was nearly the same; similar values were obtained with the analogue system, although greater fluctuations in the data were observed in contrast with the data collected using photon counting. During the experiments a slit width of 0.100 mm was used; this corresponds to a theoretical half-band width of 0.08 nm.

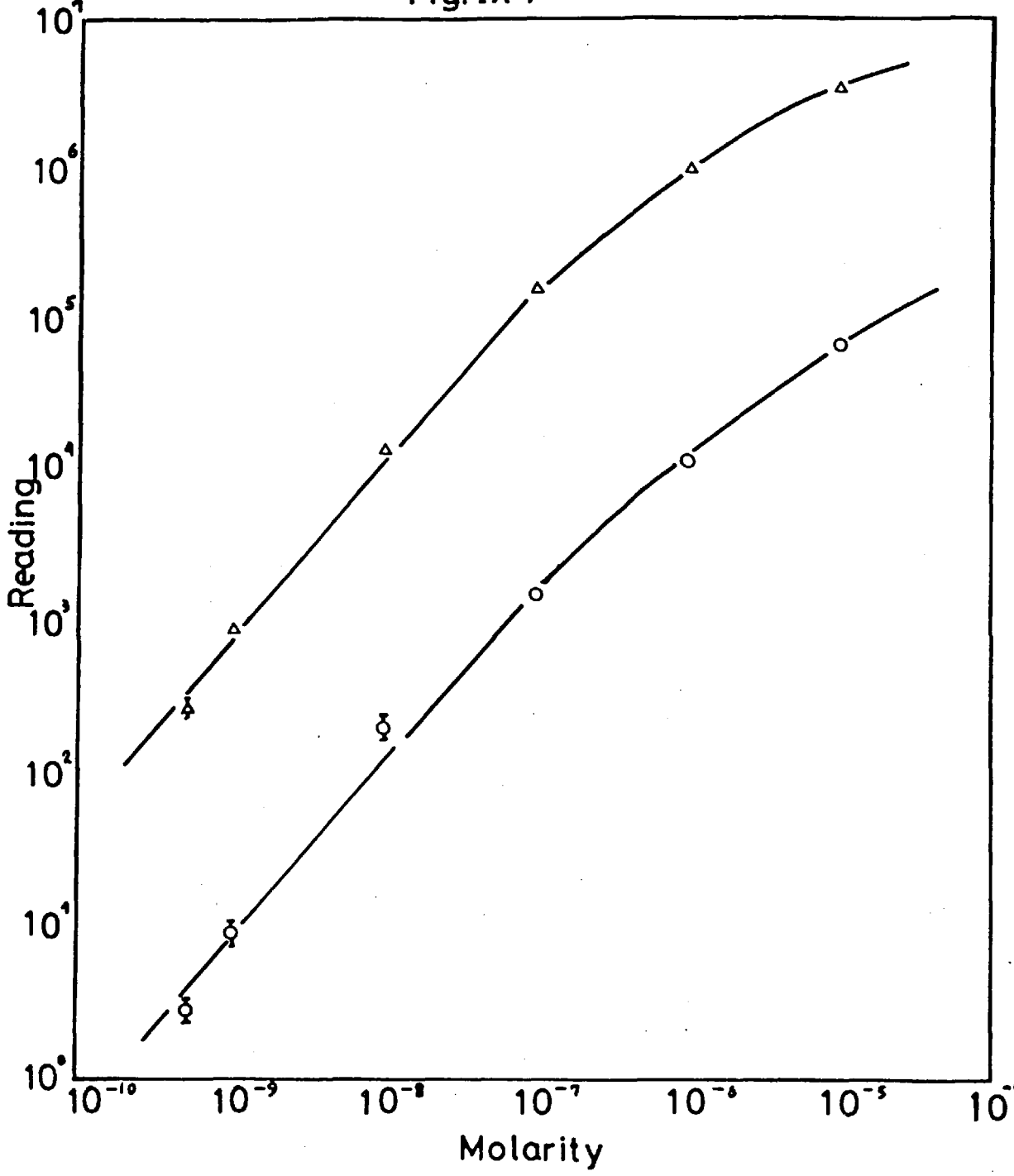
Analytical experiments

3,4-benzopyrene study

Using the optimum experimental conditions, 3,4-benzopyrene was studied using both analogue and photon counting. A counting time of 5 seconds was used in most of the experiments unless otherwise stated. Figure IX - 7 shows the calibration curves (reading in mV when analogue, in total counts per 5 seconds when photon counting). A very similar range of linearity was observed with both systems; the curves are nearly linear from 1 x 10^{-9}M to 1 x 10^{-7}M which agrees with the previous observations of Jäger and Lugrová[111]. The curvature above 1 x 10^{-7}M can be attributed to an inner-filter effect as the excitation spectrum of 3,4-benzopyrene has a maximum at 402.87 nm in n-octane at 77 K[72].

The readings for the analogue measurements were taken using the net difference between the readings at the maximum (at 403.0 nm) and the readings at the background at 400.0 nm. An exception was made in the cases where the concentration was less than 1 x 10^{-8}M, when a base line method was employed, as the peak of 3,4-benzopyrene is located in a shoulder of a scattered mercury line (at 404.75 nm). In these cases the net value was determined after a base line was traced

Fig. IX-7



3,4 Benzopyrene in n octane.
Photon count(△) Analogue(○) 5 sec. counting time.

between the 'valleys' at 402.0 and 403.25 nm.

The photon counting readings, plotted also in Figure IX - 7, are also net values. At higher concentrations ($> 1 \times 10^{-8}$M) the values were taken from the differences between the photon counting readings at 402.0 (or 400.0) and the readings at 403.0 nm. At low concentrations ($\leq 1 \times 10^{-8}$M) the average readings obtained between the values at 402.0 and 403.25 nm was subtracted from the reading at 403.0 nm. Although the curves suggest an increase in sensitivity by the use of photon counting, the determination of the signal-to-noise ratio shows for both systems the same value for the limit of detection (5×10^{-10}M). Table IX - 3 shows the variation of relative standard deviation (RSD) with concentation.

TABLE IX - 3

3,4-Benzopyrene

Concentration (Molarity)	5×10^{-10}	1×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}	1×10^{-5}
Analogue (RSD,%)	32.3	24.6	5.3	3.9	1.6	1.4
Photon Counting (RSD,%)	46.0	2.0	1.3	0.55	0.18	0.70

At concentrations higher than 5×10^{-10}M the photon counting method shows a much better precision; the limits of detection of both methods however are the same. If the counting time is longer an increase in the SNR (using equation IX - J) is observed at concentrations higher than the limit of detection; at the limit of detection there is only a slight increase (Figure IX - 8).

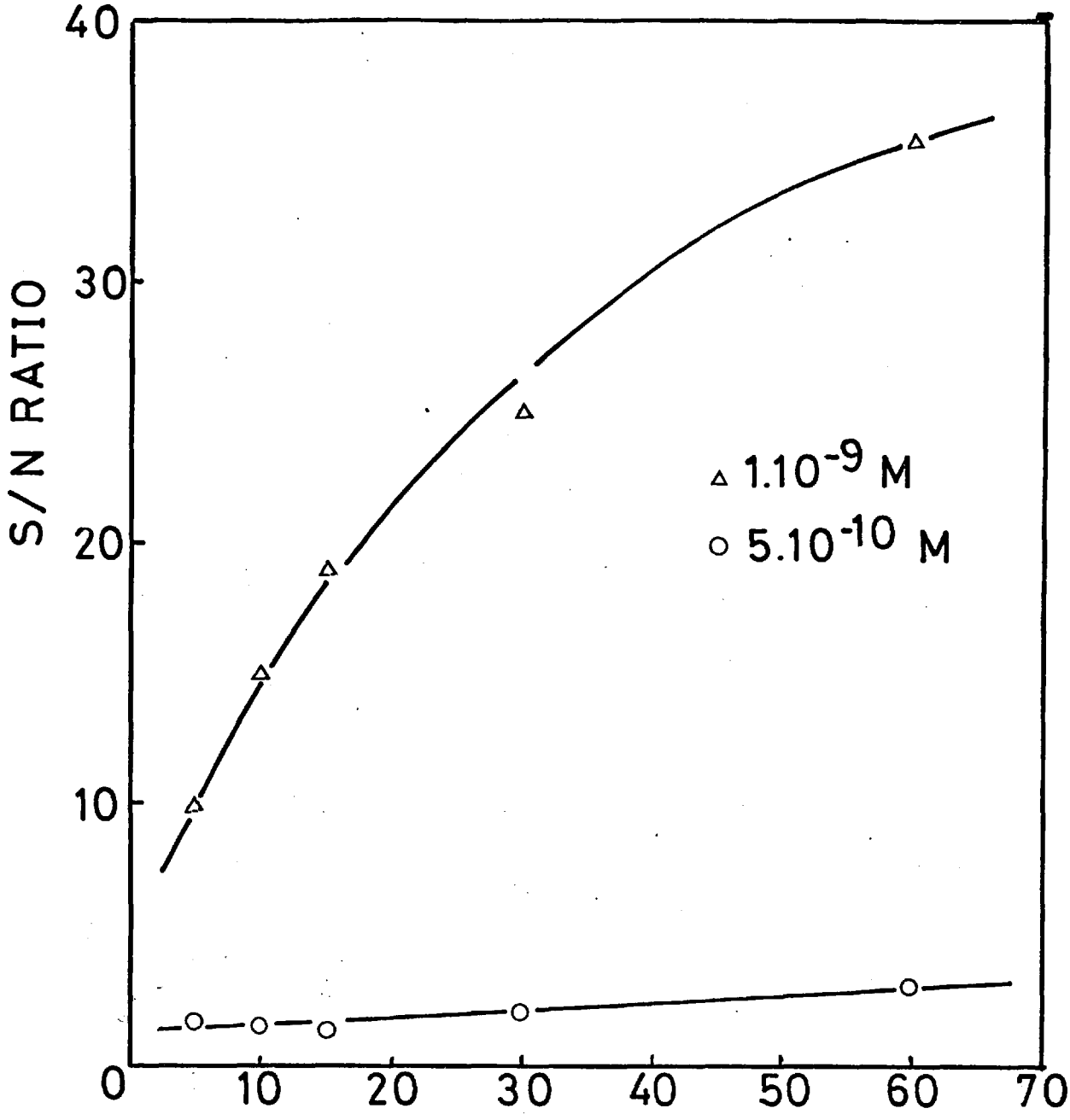


Fig. IX-8

3,4,9,10-Dibenzopyrene study

3,4,9,10-Dibenzopyrene was also studied and curves using the analogue and photon counting techniques can be seen in Figure IX - 9. The curves are both linear between 1×10^{-9} and 1×10^{-6} M, and the precision of the data is shown in Table IX - 4.

TABLE IX - 4

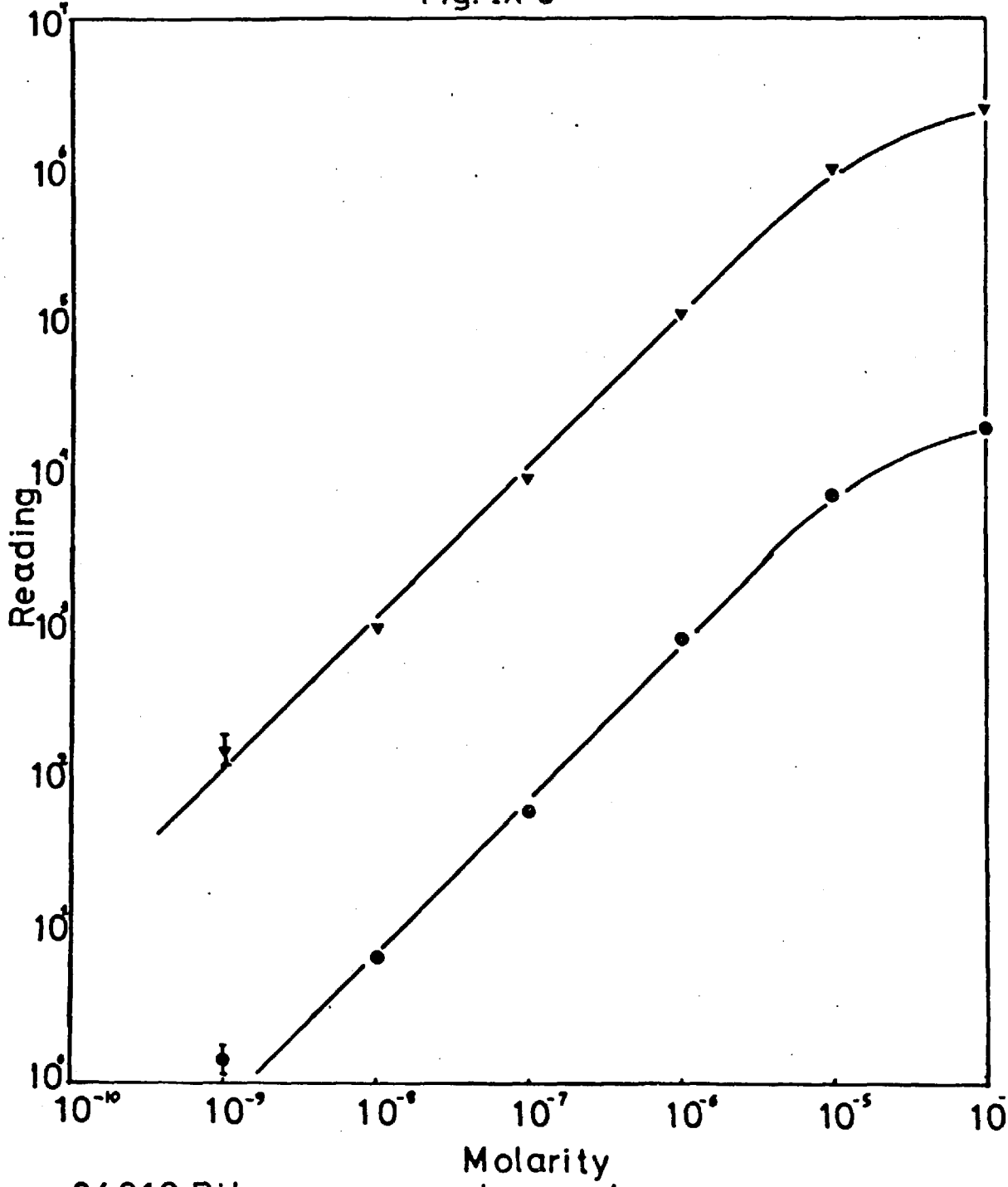
3,4,9,10-Dibenzopyrene

Concentration (Molarity)	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$
Analogue (RSD,%)	33	6.2	4.4	4.0	2.2	1.1
Photon Counting (RSD,%)	44	5.0	0.6	0.3	0.6	0.9

More precise values are again obtained with photon counting at concentration $> 1 \times 10^{-8}$M, but the limit of detection is the same for both system (1×10^{-9}M). The net readings were obtained in the analogue and in photon counting by subtracting the reading at 428.0 nm from the reading at 431.70 nm (the maximum). At low concentration ($< 1 \times 10^{-8}$M), again it was necessary, due to a slope in the scattered background, to establish a baseline between 428.0 nm and 433.0 nm.

Much lower background interference was observed ($\sim$200 $csec^{-1}$) in the case of 3,4,9,10-dibenzopyrene, due to the absence of direct interference from scattered mercury line radiation and of a scattered continuum present, due to the fact that the interference filter employed (300 nm maximum) has its peak transmission far from the

Fig. IX-9



3,4,9,10 Dibenzopyrene in n octane.
Photon count.(▾) Analogue(●) 5 sec. counting time.

maximum emission of 3,4,9,10-dibenzopyrene (431.70 nm) in comparison with the maximum emission of 3,4-benzopyrene (403.0 nm).

Application of photon counting in quantitative analysis

An evaluation of photon counting applied to quantitative analysis was made using a sample of used lubricant oil which was also analysed using the conventional record trace method. The combined internal standard-standard addition method was used; the net readings at the maximum for 3,4-benzopyrene were related to the net readings for coronene ($I_{444.55} - I_{443.0}$) and were plotted. The result obtained was compared with the result obtained using the analogue method and an almost similar value was found (analogue 3.2×10^{-7} M *vs* photon counting 2.7×10^{-7}M), although the time consumed in the photon counting experiment was much longer, as the 'baseline' method applied to photon counting requires that data be taken at each wavelength and the average of each set of numbers obtained be evaluated.

3. Signal averaging

The simplest method of eliminating or minimising noise, consists of the use of a d.c. amplifier with a long read out time constant (using a RC low pass filter) which has been already referred to in the introduction to this Chapter. A d.c. detection system is, however, subject to output fluctuations due to variations of the gain of the amplifier, high frequency noise in the electronic circuit in general and very low

frequency noise (drift). In order to increase the SNR in wavelength scanning spectrometry using a d.c. amplification and RC filter it is necessary to establish a compromise between response time and scan velocity. Noise is also minimised by the use of the d.c. integration system already described.

Another method,which is finding increasing utility for discrimination of signal from noise,is to modulate the radiation studied in the experiment and apply the signal at the detector to a narrow band amplifier which is operated synchronously with the modulation. This type of device discriminates against noise, not only on the basis of the frequency but also on the basis of phase; this technique is called lock-in(or phase sensitive) amplification[234] and has been successfully used in analytical techniques such as atomic absorption and fluorescence spectrometry.

When the signal is not periodic but can be made repeatable the SNR can be enhanced by the techniques of signal averaging (sometimes also called time averaging or emsemble averaging)[234,241-2]. The technique of signal averaging is based in the instrumental superposition of a number of single spectrum sweeps, by sampling each signal in the same way (same spectrum range) and in the same corresponding times, and storing the array of measurement in a multichannel memory. This requires the use of a synchronizing signal or pulse (a trigger link) between the experiment and the measurement in order to actuate the sampling system. The trigger systems used may be either internal or external[234,241-2].

The storage device normally used is commercially available in the form of a multichannel analyzer which has been used in mass spectrometry, Mössbauer spectroscopy and for measurement of bioelectric

phenomena and in signal averaging applied to nuclear magnetic resonance[243].

Signal-to-noise

It will be expected that with the number of repetitions of the spectrum in a certain range, the signal would increase linearly, whereas the random fluctuation will algebraically add to zero. In reality the signal and other interferences which are in phase with the signal will add coherently in the register while the random noise will average less rapidly and to a small value in comparison with the signal.

According to Klein and Barton[243], when signal averaging is used, theoretically it is expected that the signal should increase linearly in the register with the numbers of records sampled (N), whereas the random fluctuation will add as the square root of this number ($\sqrt{N}$). Consequently the SNR increases proportionally to the square root of the number of scans - $\sqrt{N}$ (sometimes called the '$\sqrt{N}$ law'). This assumes that if S is the average of value of a large number of measurements N, then the noise n may be represented by the root-mean-square (rms) deviation from the mean[244]

$$n = \left[\left(\frac{1}{N}\right) \sum_j (S_j - \bar{S})^2 \right]^{\frac{1}{2}} \qquad \text{IX - K}$$

If, for instance, the number of measurements are doubled this would not change the average but it would double the accumulated signal; the noise however would decrease by a factor of $\sqrt{2}$ (from equation IX - K). Under these conditions the SNR will be improved in a $\sqrt{N}$ proportion.

Ernst[242] undertook a theoretical analysis of the signal averaging

and observed that only in special cases does the SNR improve exactly in accordance with the $\sqrt{N}$ law. If the noise shows no coherence between successive scans (white random noise) the SNR increases linearly with the square root of the number of scans. On the other hand, if the noise shows partial coherence between successive sweeps, the enhancement of the SNR will be slower or non-existent.

If the noise is harmonic with the scanning time the SNR cannot be improved unless the scanning time is changed. According to Hieftje[234,241] the sampling of the signal recordings must meet certain criterion: it must be sampled at a frequency at least twice as great as its highest frequency component. If the frequency is greater than twice the highest signal frequency, however, no additional information will be obtained but more noise will be included than is desirable. With noise of low frequency (drift) Ernst[242] demonstrated that signal averaging can be even more effective than for white noise. Usually the type of noise encountered in experimental conditions is a combination of white noise and 1/f noise. Other types of noise will affect the SNR in different ways depending on the possibility of it being coherent or harmonic with the sampling time. Normally as the impulses are random processes they will affect only a few sweeps during the averaging and consequently not contribute to a decrease in the SNR.

Unwanted distortion noise or unwanted signals such as d.c. offset, dark current or background are inevitably mixed with the signal and a blank must be run at the same conditions in order to be sure of the signal present[244].

Signal averaging and filtering

The application of signal averaging to nuclear magnetic resonance calls attention to the fact that signal averaging is complementary to filtering methods; i.e. does not replace them[242]. Signal averaging is a method which deals in the time domain in contrast with filtering which is an operation in the frequency domain. Filtering is based on the different spectral properties of signal and noise while signal averaging depends on differences in the properties of coherence of a repetitive signal and noise. Ernst[245] believes that a combination of both methods produces the optimum results.

A filtering device - such as a single section, low pass RC filter - can be used in front of the sampling system, although it is more convenient to include also a filtering device in the sampling process by taking an average of a signal over a limited time interval, the most common procedure being to integrate during the gating time, τ.[244-5] This filter system can, in reality, act as a pre-average device during the gate time; this lowers the high frequency noise in proportion to $\sqrt{\tau}$[244].

Signal averaging experiments

Preliminary experiments with signal averaging were made using a signal processing system.(Unimac 4000, Data Laboratories Ltd., Mitcham, England). (see Plate IX - 1,b). This apparatus has an on-line digital processing system for the storage and analysis of waveforms

in both the time and frequency domains. The instrument is modular and has four basic units. The first is a memory with 1024 words. Another unit consists of a sweep timer, which generates timing pulses to control the rate at which the data is sampled during analysis. Delay times after each sweep (from 1 μsec to 999 msec) and before each sweep (from 1 msec to 999 sec) may be selected in increments of units. A programme unit is provided in which not only single channel averaging is possible but also multichannel averaging, as four single channel inputs are available and these can be averaged simultaneously.

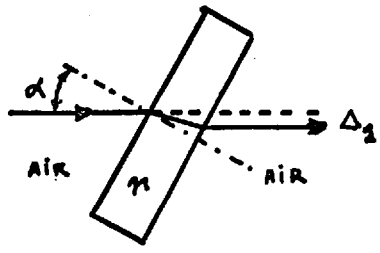
A display control module provides the possibility of setting the number of sweeps to be counted, in 2^n intervals from $1(2^0)$ to 16384 (2^{14}). In the same control module there are commands which control signals to an osciloscope or an XY plotter. Another module also contains controls to tape punch.

Different modes of trigger are possible: an automatic mode which actuates another sweep after the end of each sweep, and an external trigger mode which is actuated from an outside signal; the external trigger mode was used in all our experiments.

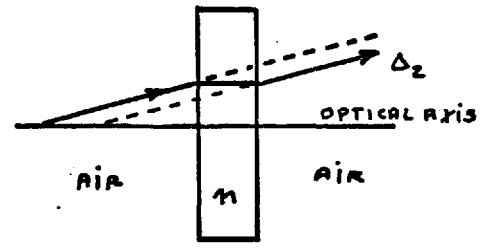
To produce the repetitive scans an oscillating transparent refractor plate mechanism, which was a modification of one proposed by William[246], Roldan[247] and Snelleman et al[248] was used by us and was located just behind the entrance slit of the monochromator. A frequency of oscillation of 5 Hz was used in all experiments.

Other methods have been used by other workers to scan repetitively or to modulate the wavelength, such as by means of a oscillating mirror, by oscillation of the entrance or exit slit, and even, at low modulation frequencies, to oscillate the monochromator directly[249].

The amount of lateral displacement of the light beam when a refractor plate is in use and consequently the range of spectrum being



lateral displacement



longitudinal displacement

repeated is given by

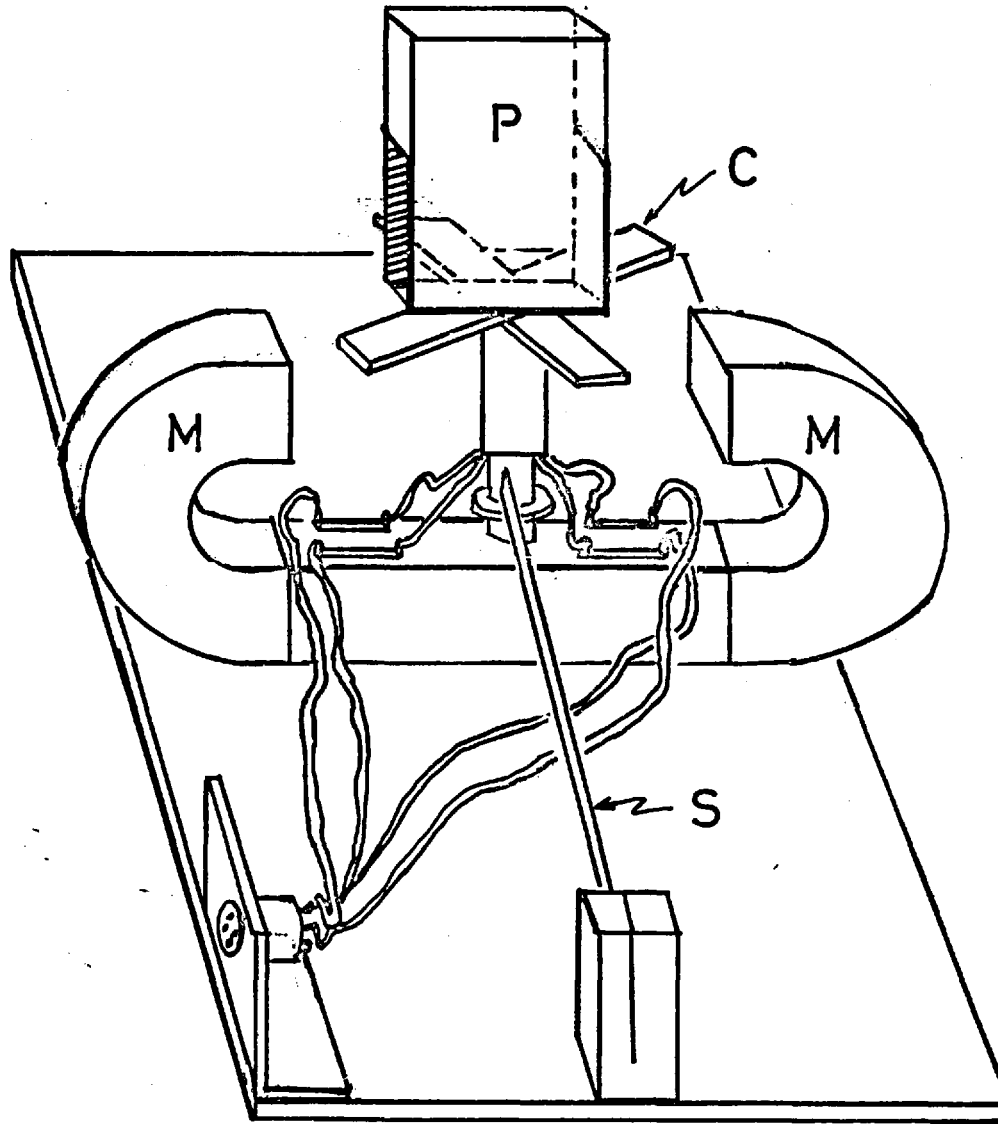
$$\Delta_1 \simeq [t\alpha(n - 1)/n] \qquad \text{IX - L}$$

where t is the thickness of the plate, α-radians in air and n is the index of refraction of the refractor plate material. Either by increasing the thickness of the plate 't' or the amplitude of the oscillation (and to a lesser extent the index of refraction), the spectrum range can be increased. The beam, in addition to being displaced laterally (and parallel) to its initial direction, is also shifted longitudinally. This displacement is given by[248]

$$\Delta_2 = t(n - 1)/n \qquad \text{IX - M}$$

where t and n have the same meanings as in equation IX - L.

Initially experiments were made using as refractor a quartz



MECHANICAL OSCILLATOR
P-oscillating plate. C-cross with coil.
M-permanent magnets. S-spring

Fig. IX-10

absorption cell (10 mm path length), which was filled with different liquids such as water ($n_D^{20°}$ = 1.333), n-octane ($n_D^{20°}$ = 1.3975), n-decanol ($n_D^{20°}$ = 1.4372). This system, although it produced a repetitive scan, had only a small range of the order of 1.0 - 1.2 nm - which was not sufficient for our purposes. Later the quartz cell was replaced by a Perspex - poly(methyl methacrylate), $n_D^{20°}$ = 1.490 - block, of 25 mm path length, which gave the possibility of repetitively scanning of a range of 2 - 3nm.

The Perspex refractor plate used was transparent from 345 nm throughout the visible region. Figure IX-10 shows the design of the oscillator with the Perspex refractor in use.

The oscillator was driven by a oscillator circuit, similar to one published by Snelleman et al[248] and the amplitude of oscillation was controlled through a knob in this unit. Both oscillator and driven unit were constructed by the Electrical workshop. Calibration of the scan range was measured using a mercury triplet at 365 - 366 nm (365.01, 365.47 and 366.33 nm) from an Hg lamp source. Figure IX - 11a shows a typical calibration spectrum where the 365.01 and 365.47 nm lines are present.

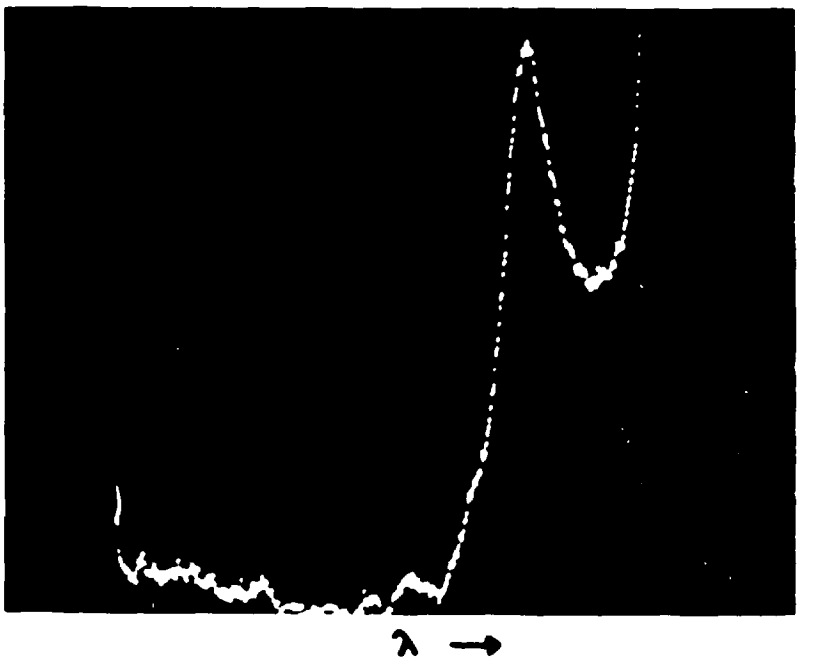
In earlier experiments the sample was replaced by a polished aluminium plate freshly covered with magnesium oxide used as a reflector; later the polycrystalline frozen matrix itself was used as a reflector to calibrate thesscan range.

Using 3,4-benzopyrene in n-octane, and coronene in n-hexane, several experiments were undertaken. In all experiments the copper cell described in Chapter VII was used; this proved extremely convenient, principally when long averaging times were used. These preliminary experiments revealed that no sensitivity improvement was observed by the



a

(mercury lines)



b

($5X10^{-9}$ M 3,4-benzopyrene)

direct introduction of the output from the photomultiplier (EMI 6256S) into one of the inputs of the signal averager, even using long averaging times (one hour or more). Later a pre-amplifier was constructed with a gain of ~ 50 (Figure IX - 12), to permit the study of weaker signals Low concentrations of 3,4-benzopyrene (1×10^{-8}M, 1×10^{-9} M) in n-octane were averaged with time for as long as one hour and the signal obtained viewed at an oscilloscope (Hewlett Packard Model 175A - Plate IX - 1,a) and photographed (see Figure IX - 11b) or point plotted using a recorder (Servoscribe Model RE 511.20). These averaged signals were compared vs potentiometric recorder traces obtained using the RCA WV-84C microameter as a d.c. amplifier. Although the signal averager seems to improve the problem caused by drift, some high frequency noise component, which apparently was coherent, was not averaged. Attempts were then made to improve the situation using low pass filters by introductions into the pre-amplifier of a set of capacitors (Figure IX - 12). With this modification it was possible to reject frequencies

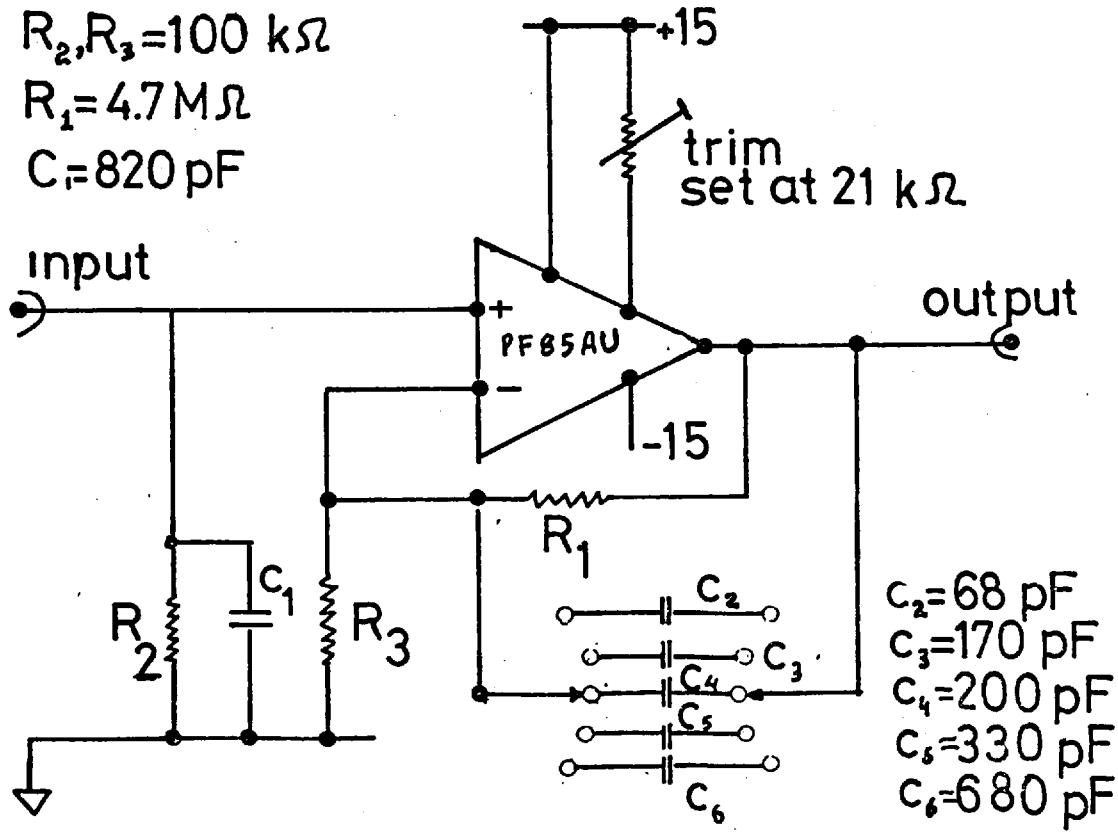


Fig. IX-12

higher than 800,350,285,170 or 85 Hz, by previous selection with the introduction in the circuit (through the use of amultiple switch) of a different capacitor. With the rejection at 85 Hz some distortion of the spectrum was observed, although the spectrum was much less noisy. Using the 170 Hz rejection filter the improvement in the SNR was on the order of 1.8 - 2.2 times in comparison with the analogue trace, although was necessary to average,for long periods (ca. one hour). Table IX - 5 shows the results for 2.5×10^{-8} M, coronene. The signal-to-noise ratio using the recorder trace was 4.7. The SNR values for the signal averaging experiments were estimated from the recorder plots.

TABLE IX - 5

Filter (Hz)	800	350	170	85
Relative signal intensity	1.0	1.0	1.0	0.8
SNR	2.7	9.7	11.0	15.7

As the signal averager provides the possibility of better resolution by using a very narrow slit other experiments were also made, although without success, to observe fine structure in the emission maxima of coronene in n-hexane and 3,4-benzopyrene in n-octane, both at 80 K. Also a study of the influence of concentration in the half-band width of such compounds was carried out, however no effect was observed.

The present signal averaging system however needs, further improvement, such as a better repetitive scanning device. The system used in this work suffers from the limited lifetime of the phosphor

bronze spring, due to the large amplitude of oscillation used during the averaging process. Another improvement that will probably bring the signal averager to its full application is the use of an integration circuit that integrates during the gating time. Since writing this thesis a better resolution has been observed which suggests that further optimization of the whole system will be helpful.

Conclusion

D.c. integration and photon counting can be used with success in quasi-linear luminescence analysis. Although direct comparison was not possible between d.c. integration and photon counting as different instruments were used at different times d.c. integration seems to be the first choice if low cost is essential. The major problem in both cases is the presence of a strong background originating from excitation scatter, a problem which is probably as old as luminescence: Nichols and Merrit[6] in a paper in 1911 stated '... special precautions are necessary to avoid the blending of the excitation light with that emitted by the fluorescent substance'!

In the case of d.c. integration a backing-off device was available and used to compensate the continuum present, however, no compensation is possible with the scattered lines. In the present case to use, either d.c. integration or photon counting it is necessary to obtain a certain number of readings at certain wavelength and to find an average of each set of readings. This process is time-consuming. Electronic data processing is, however, possible and probably will be available for such systems, when such time consuming operations can be avoided. Also, a commercial instrument company recently announced the availability of a photon counter which is, apparently, capable of providing a plotted spectrum with much improved signal-to-noise ratio.

If further improvements are made in the present signal averaging system undoubtedly the signal to noise ratio will increase. Signal averaging has, in the present study, the advantage of providing the possibilty to observe a certain range of spectra in an oscilloscope during the averaging process and also with this particular instrument the possibility of storing the spectrum either on punch tape (which can be used for further data treatment or simply plotted when necessary), or it may be directly plotted using a potentiometric recorder.

CHAPTER X

Conclusions

The application of the Shpol'skii effect for analytical purposes has been examined in this thesis. The method seems to be extremely specific and sensitive, although it is not universal, as many compounds do not produce the Shpol'skii effect.

There are cases where the sample can be analysed without previous clean-up or separation processes; where a separation is necessary, the use of high pressure liquid chromatography together with quasi-linear spectrometry will probably provide an excellent combination for the analysis of organic compounds, in particular polycyclic aromatic hydrocarbons and some heterocyclics.

With some of the compounds studied most of the quenching observed seems to be due to the presence of an inner-filter effect. Eventually it will be interesting to see the influence of interferences on the lifetime of the excited states as the lifetime is influenced by the environment of the analyte molecule.

Tetrahydrofuran was found to be a suitable matrix for the production of quasi-linear luminescence, although this matrix has the usual limitations as a quasi-linear matrix, should the occasion arise it can be used in analysis if a particular compound present in an aqueous sample, is soluble and produces quasi-linear luminescence in THF. Care, however, must be taken with the problems caused by the presence or generation of peroxide. On the other hand, the determination of the crystallographic structure of tetrahydrofuran at 77 K and the comparison with the well known crystallographic structures of the n-paraffin solvents may contribute to the understanding of the interesting phenomenon, of the Shpol'skii effect.

In Chapter VIII several compounds were tested in THF and for the reasons already discussed most of them failed to produce quasi-linear luminescence. If, the amount of compound being analysed is enough for a reaction to be carried out to produce a product, which is non-polar and consequently less interactive this compound may produce quasi-linear luminescence. Possibly this derivatization technique may be used as a method of identification and determination or even as a method of structure characterization. For trace analysis, when very small amounts of compound are present, and in cases where there are side reactions or non-specificity in the reaction, this approach may be difficult to apply.

The use of stronger and stabler sources for excitation will, obviously, improve the sensitivity of the method. A tunable dye laser will probably be the best solution, when it will be expected that, together with the enhancement in signal intensity, the problem of light scattering, usually encountered with the use of mercury and xenon excitation, will be smaller. The increase in the signal-to-background ratio with the use of a tunable dye laser will improve and reinforce the possibility of using the techniques of signal processing tested in this thesis (d.c. integration, photon counting and signal averager). As the current trend is suggesting, vidicon multichannel detectors will be more and more employed in analytical spectrometry;[250,251] as soon as the UV sensitivity of this type of detectors is enhanced it will be interesting to test these for quasi-linear luminescence spectrometry.

The use of different types of monochromators with different optical assemblies, apertures and resolution lead us to the conclusion that for vibrational analysis,

or for fundamental studies, it is necessary to use monochromator of high resolution (≤ 1.0 nm mm^{-1}) and probably the approach of a laboratory-made modular assembly will always be the best approach. For application to Analytical Chemistry most of the fluorimeters commercially available are of low resolution and if quasi-linear luminescence spectrometry is to be used a new generation of instruments must be designed; it is important, however, to take into consideration that not all compounds will produce the Shpol'skii effect. In consequence a compromise must be established between the utility of such an instrument, the resolution of the monochromator and the cost. A reciprocal dispersion of 1.0 - 2.0 nm mm^{-1} (and a low f. number) will be satisfactory for routine purchases.

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Publications

1. G.F. Kirkbright and C.G. de Lima 'The Detection and Determination of Polynuclear Aromatic Hydrocarbons by Luminescence Spectrometry Utilising the Shpol'skii Effect at 77 K'.Analyst, 99, 338(1974).
2. G.F. Kirkbright and C.G. de Lima 'Quasi-Linear Fluorescence and Phosphorescence of some Aromatic and Heterocyclic Compounds in Tetrahydrofuran at 77 K'. Accepted for publication in Chemical Physics Letters.